

* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 12:03:43 ON 07 OCT 2002

=> fil .bec,canc
COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
0.21	0.21

FULL ESTIMATED COST

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
ESBIOBASE, BIOTECHNO, WPIDS, CANCERLIT' ENTERED AT 12:04:01 ON 07 OCT 2002
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s sulfotransferase#

FILE 'MEDLINE'

L1 1892 SULFOTRANSFERASE#

FILE 'SCISEARCH'

L2 2286 SULFOTRANSFERASE#

FILE 'LIFESCI'

L3 574 SULFOTRANSFERASE#

FILE 'BIOTECHDS'

L4 77 SULFOTRANSFERASE#

FILE 'BIOSIS'

L5 2538 SULFOTRANSFERASE#

FILE 'EMBASE'

L6 2056 SULFOTRANSFERASE#

FILE 'HCAPLUS'

L7 2789 SULFOTRANSFERASE#

FILE 'NTIS'

L8 14 SULFOTRANSFERASE#

FILE 'ESBIOBASE'

L9 727 SULFOTRANSFERASE#

FILE 'BIOTECHNO'

L10 893 SULFOTRANSFERASE#

FILE 'WPIDS'

L11 55 SULFOTRANSFERASE#

FILE 'CANCERLIT'

L12 465 SULFOTRANSFERASE#

TOTAL FOR ALL FILES

L13 14366 SULFOTRANSFERASE#

=> s l13(5a) phenol?

FILE 'MEDLINE'

29635 PHENOL?

L14 259 L1 (5A) PHENOL?

FILE 'SCISEARCH'

56527 PHENOL?

L15 527 L2 (5A) PHENOL?

FILE 'LIFESCI'
15480 PHENOL?
L16 93 L3 (5A) PHENOL?

FILE 'BIOTECHDS'
10598 PHENOL?
L17 5 L4 (5A) PHENOL?

FILE 'BIOSIS'
61999 PHENOL?
L18 393 L5 (5A) PHENOL?

FILE 'EMBASE'
27661 PHENOL?
L19 247 L6 (5A) PHENOL?

FILE 'HCAPLUS'
327704 PHENOL?
L20 492 L7 (5A) PHENOL?

FILE 'NTIS'
6558 PHENOL?
L21 0 L8 (5A) PHENOL?

FILE 'ESBIOBASE'
20062 PHENOL?
L22 114 L9 (5A) PHENOL?

FILE 'BIOTECHNO'
9007 PHENOL?
L23 109 L10 (5A) PHENOL?

FILE 'WPIDS'
107702 PHENOL?
L24 3 L11 (5A) PHENOL?

FILE 'CANCERLIT'
3785 PHENOL?
L25 41 L12 (5A) PHENOL?

TOTAL FOR ALL FILES
L26 2283 L13 (5A) PHENOL?

=> s l13 and pst
FILE 'MEDLINE'
1595 PST
L27 152 L1 AND PST

FILE 'SCISEARCH'
1803 PST
L28 151 L2 AND PST

FILE 'LIFESCI'
636 PST
L29 60 L3 AND PST

FILE 'BIOTECHDS'
122 PST
L30 1 L4 AND PST

FILE 'BIOSIS'
2520 PST
L31 181 L5 AND PST

FILE 'EMBASE'
1311 PST
L32 196 L6 AND PST

FILE 'HCAPLUS'
2107 PST
L33 178 L7 AND PST

FILE 'NTIS'
116 PST
L34 0 L8 AND PST

FILE 'ESBIOBASE'
419 PST
L35 60 L9 AND PST

FILE 'BIOTECHNO'
619 PST
L36 81 L10 AND PST

FILE 'WPIDS'
396 PST
L37 0 L11 AND PST

FILE 'CANCERLIT'
264 PST
L38 28 L12 AND PST

TOTAL FOR ALL FILES
L39 1088 L13 AND PST

=> s 126 or 139

FILE 'MEDLINE'
L40 299 L14 OR L27

FILE 'SCISEARCH'
L41 542 L15 OR L28

FILE 'LIFESCI'
L42 108 L16 OR L29

FILE 'BIOTECHDS'
L43 6 L17 OR L30

FILE 'BIOSIS'
L44 426 L18 OR L31

FILE 'EMBASE'
L45 330 L19 OR L32

FILE 'HCAPLUS'
L46 518 L20 OR L33

FILE 'NTIS'
L47 0 L21 OR L34

FILE 'ESBIOBASE'
L48 131 L22 OR L35

FILE 'BIOTECHNO'
L49 139 L23 OR L36

FILE 'WPIDS'
L50 3 L24 OR L37

FILE 'CANCERLIT'
L51 52 L25 OR L38

TOTAL FOR ALL FILES
L52 2554 L26 OR L39

=> s hormone(3a)depend?

FILE 'MEDLINE'
205775 HORMONE
808828 DEPEND?
L53 7064 HORMONE (3A) DEPEND?

FILE 'SCISEARCH'
184453 HORMONE
1100019 DEPEND?
L54 3332 HORMONE (3A) DEPEND?

FILE 'LIFESCI'
46497 HORMONE
256513 DEPEND?
L55 1170 HORMONE (3A) DEPEND?

FILE 'BIOTECHDS'
6692 HORMONE
17173 DEPEND?
L56 54 HORMONE (3A) DEPEND?

FILE 'BIOSIS'
410263 HORMONE
888737 DEPEND?
L57 5449 HORMONE (3A) DEPEND?

FILE 'EMBASE'
250008 HORMONE
768339 DEPEND?
L58 4946 HORMONE (3A) DEPEND?

FILE 'HCAPLUS'
233958 HORMONE
1936328 DEPEND?
L59 5168 HORMONE (3A) DEPEND?

FILE 'NTIS'
1868 HORMONE
132487 DEPEND?
L60 57 HORMONE (3A) DEPEND?

FILE 'ESBIOBASE'
50074 HORMONE
273543 DEPEND?
L61 1317 HORMONE (3A) DEPEND?

FILE 'BIOTECHNO'
69964 HORMONE
223445 DEPEND?
L62 1804 HORMONE (3A) DEPEND?

FILE 'WPIDS'
12042 HORMONE
261064 DEPEND?
L63 478 HORMONE (3A) DEPEND?

FILE 'CANCERLIT'

49836 HORMONE
177688 DEPEND?
L64 5136 HORMONE(3A) DEPEND?

TOTAL FOR ALL FILES
L65 35975 HORMONE(3A) DEPEND?

=> s 152 and 165
FILE 'MEDLINE'
L66 0 L40 AND L53

FILE 'SCISEARCH'
L67 1 L41 AND L54

FILE 'LIFESCI'
L68 0 L42 AND L55

FILE 'BIOTECHDS'
L69 0 L43 AND L56

FILE 'BIOSIS'
L70 0 L44 AND L57

FILE 'EMBASE'
L71 0 L45 AND L58

FILE 'HCAPLUS'
L72 1 L46 AND L59

FILE 'NTIS'
L73 0 L47 AND L60

FILE 'ESBIOBASE'
L74 0 L48 AND L61

FILE 'BIOTECHNO'
L75 0 L49 AND L62

FILE 'WPIDS'
L76 0 L50 AND L63

FILE 'CANCERLIT'
L77 0 L51 AND L64

TOTAL FOR ALL FILES
L78 2 L52 AND L65

=> s 152 and risk
FILE 'MEDLINE'
516194 RISK
L79 5 L40 AND RISK

FILE 'SCISEARCH'
350263 RISK
L80 14 L41 AND RISK

FILE 'LIFESCI'
49188 RISK
L81 4 L42 AND RISK

FILE 'BIOTECHDS'
1915 RISK
L82 0 L43 AND RISK

FILE 'BIOSIS'
315254 RISK
L83 7 L44 AND RISK

FILE 'EMBASE'
458271 RISK
L84 6 L45 AND RISK

FILE 'HCAPLUS'
93241 RISK
L85 14 L46 AND RISK

FILE 'NTIS'
38894 RISK
L86 0 L47 AND RISK

FILE 'ESBIOBASE'
96510 RISK
L87 6 L48 AND RISK

FILE 'BIOTECHNO'
42047 RISK
L88 2 L49 AND RISK

FILE 'WPIDS'
60837 RISK
L89 0 L50 AND RISK

FILE 'CANCERLIT'
119580 RISK
L90 3 L51 AND RISK

TOTAL FOR ALL FILES
L91 61 L52 AND RISK

=> s 178 or 191
FILE 'MEDLINE'
L92 5 L66 OR L79

FILE 'SCISEARCH'
L93 15 L67 OR L80

FILE 'LIFESCI'
L94 4 L68 OR L81

FILE 'BIOTECHDS'
L95 0 L69 OR L82

FILE 'BIOSIS'
L96 7 L70 OR L83

FILE 'EMBASE'
L97 6 L71 OR L84

FILE 'HCAPLUS'
L98 14 L72 OR L85

FILE 'NTIS'
L99 0 L73 OR L86

FILE 'ESBIOBASE'
L100 6 L74 OR L87

FILE 'BIOTECHNO'

```

L101          2 L75 OR L88

FILE 'WPIDS'
L102          0 L76 OR L89

FILE 'CANCERLIT'
L103          3 L77 OR L90

TOTAL FOR ALL FILES
L104          62 L78 OR L91

=> s l104 not 1999-2002/py
FILE 'MEDLINE'
      1798929 1999-2002/PY
L105          2 L92 NOT 1999-2002/PY

FILE 'SCISEARCH'
      3578296 1999-2002/PY
L106          8 L93 NOT 1999-2002/PY

FILE 'LIFESCI'
      360388 1999-2002/PY
L107          2 L94 NOT 1999-2002/PY

FILE 'BIOTECHDS'
      53832 1999-2002/PY
L108          0 L95 NOT 1999-2002/PY

FILE 'BIOSIS'
      1949185 1999-2002/PY
L109          3 L96 NOT 1999-2002/PY

FILE 'EMBASE'
      1596024 1999-2002/PY
L110          3 L97 NOT 1999-2002/PY

FILE 'HCAPLUS'
      3421986 1999-2002/PY
L111          5 L98 NOT 1999-2002/PY

FILE 'NTIS'
      62667 1999-2002/PY
L112          0 L99 NOT 1999-2002/PY

FILE 'ESBIOBASE'
      1032395 1999-2002/PY
L113          3 L100 NOT 1999-2002/PY

FILE 'BIOTECHNO'
      434498 1999-2002/PY
L114          1 L101 NOT 1999-2002/PY

FILE 'WPIDS'
      3010571 1999-2002/PY
L115          0 L102 NOT 1999-2002/PY

FILE 'CANCERLIT'
      329161 1999-2002/PY
L116          2 L103 NOT 1999-2002/PY

TOTAL FOR ALL FILES
L117          29 L104 NOT 1999-2002/PY

=> dup rem l117

```

PROCESSING COMPLETED FOR L117

L118 12 DUP REM L117 (17 DUPLICATES REMOVED)

=> d tot

L118 ANSWER 1 OF 12 HCAPLUS COPYRIGHT 2002 ACS

TI Rat, but not human, sulfotransferase activates a tamoxifen metabolite to produce DNA adducts and gene mutations in bacteria and mammalian cells in culture

SO Carcinogenesis (1998), 19(10), 1709-1713

CODEN: CRNGDP; ISSN: 0143-3334

AU Glatt, Hansruedi; Davis, Warren; Meinl, Walter; Hermersdorfer, Heino; Venitt, Stan; Phillips, David H.

AN 1998:701419 HCAPLUS

DN 130:50358

L118 ANSWER 2 OF 12 MEDLINE

DUPLICATE 1

TI Identification and characterization of cytosolic **sulfotransferases** in normal human endometrium.

SO CHEMICO-BIOLOGICAL INTERACTIONS, (1998 Feb 20) 109 (1-3) 329-39.
Journal code: 0227276. ISSN: 0009-2797.

AU Falany J L; Azziz R; Falany C N

AN 1998226408 MEDLINE

L118 ANSWER 3 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

TI Human estrogen sulfotransferase (hEST1) activities and its mRNA in various breast cancer cell lines. Effect of the progestin, promegestone (R-5020)

SO JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (SEP 1998) Vol. 66, No. 5-6, pp. 295-302.

Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
ISSN: 0960-0760.

AU Chetrite G; LeNestour E; Pasqualini J R (Reprint)

AN 1998:694924 SCISEARCH

L118 ANSWER 4 OF 12 MEDLINE

DUPLICATE 2

TI Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and **sulfotransferases**: influence on cancer susceptibility.

SO RECENT RESULTS IN CANCER RESEARCH, (1998) 154 47-85. Ref: 178
Journal code: 0044671. ISSN: 0080-0015.

AU Hengstler J G; Arand M; Herrero M E; Oesch F

AN 1999151055 MEDLINE

L118 ANSWER 5 OF 12 CANCERLIT

TI Thermostable phenolsulfotransferase (TS-**PST**) phenotype as a potential **risk** factor for colon cancer in a case-control study (Meeting abstract).

SO Proc Annu Meet Am Assoc Cancer Res, (1997) 38 A1449.
ISSN: 0197-016X.

AU Frame L T; Chou H-C; Gatlin T L; Kadlubar F F; Nowell S; Lang N P

AN 1998638449 CANCERLIT

L118 ANSWER 6 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3

TI Metabolic differences and their impact on human disease - **Sulfotransferase** and colorectal cancer

SO ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY, (DEC 1997) Vol. 4, No. 3-4, pp. 277-281.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 1382-6689.

AU Frame L T; Gatlin T L; Kadlubar F F; Lang N P (Reprint)

AN 1998:125696 SCISEARCH

L118 ANSWER 7 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)
TI XENOBIOTIC METABOLISM ENZYME GENE-EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL
AND ALVEOLAR MACROPHAGE CELLS
SO AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY, (MAR 1996)
Vol. 14, No. 3, pp. 262-271.
ISSN: 1044-1549.
AU WILLEY J C (Reprint); COY E; BROLLY C; UTELL M J; FRAMPTON M W; HAMMERSLEY
E; THILLY W G; OLSON D; CAIRNS K
AN 96:201554 SCISEARCH

L118 ANSWER 8 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)
TI IN-VITRO CONJUGATION OF BENZENE METABOLITES BY HUMAN LIVER - POTENTIAL
INFLUENCE OF INTERINDIVIDUAL VARIABILITY ON BENZENE TOXICITY
SO CARCINOGENESIS, (JUL 1995) Vol. 16, No. 7, pp. 1519-1527.
ISSN: 0143-3334.
AU SEATON M J (Reprint); SCHLOSSER P M; MEDINSKY M A
AN 95:494636 SCISEARCH

L118 ANSWER 9 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 4
TI HORMONAL INFLUENCES OF DETOXICATION IN THE RAT OVARY ON ENZYMES IN
COMPARISON WITH THE LIVER
SO BIOCHEMICAL PHARMACOLOGY, (14 FEB 1995) Vol. 49, No. 4, pp. 503-509.
ISSN: 0006-2952.
AU BECEDAS L; AHLBERG M B (Reprint)
AN 95:180024 SCISEARCH

L118 ANSWER 10 OF 12 LIFESCI COPYRIGHT 2002 CSA
TI Hormonal influences of detoxication in the rat ovary on enzymes in
comparison with the liver
SO BIOCHEM. PHARMACOL., (1995) vol. 48, no. 4, pp. 503-509.
ISSN: 0006-2952.
AU Becedas, L.; Bengtsson Ahlberg, M.*
AN 95:58202 LIFESCI

L118 ANSWER 11 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)
TI METHYLATION PHARMACOGENETICS - THIOPURINE METHYLTRANSFERASE AS A MODEL
SYSTEM
SO XENOBIOTICA, (SEP/OCT 1992) Vol. 22, No. 9-10, pp. 1055-1071.
ISSN: 0049-8254.
AU WEINSHILBOUM R M (Reprint)
AN 92:593396 SCISEARCH

L118 ANSWER 12 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)
TI POLYMORPHISMS FOR AROMATIC AMINE METABOLISM IN HUMANS - RELEVANCE FOR
HUMAN CARCINOGENESIS
SO ENVIRONMENTAL HEALTH PERSPECTIVES, (NOV 1992) Vol. 98, pp. 69-74.
ISSN: 0091-6765.
AU KADLUBAR F F (Reprint); BUTLER M A; KADERLIK K R; CHOU H C; LANG N P
AN 93:44664 SCISEARCH

=> d ab tot

L118 ANSWER 1 OF 12 HCAPLUS COPYRIGHT 2002 ACS
AB Tamoxifen increases the **risk** of human endometrial cancer and is
a potent carcinogen in rat liver, in which it produces DNA adducts and
cytogenetic damage. Nevertheless its prophylactic use against breast
cancer in healthy women is under investigation in several large trials.
To investigate whether rat hepatocarcinogenicity predicts human
hepatocarcinogenicity we used genetically engineered bacterial and
mammalian target cells to investigate how .alpha.-hydroxytamoxifen, a
major phase I metabolite of tamoxifen, is further metabolized by rat and
human phase II enzymes, sulfotransferases, to mutagenic and
DNA-adduct-forming species. We expressed rat hydroxysteroid

sulfotransferase a, a liver-specific enzyme, and corresponding human sulfotransferase in bacteria (*Salmonella typhimurium*) and in a mammalian cell line (Chinese hamster V79 cells) and tested .alpha.-hydroxytamoxifen for DNA adduct formation and mutagenicity in these systems, using unmodified cells as controls. In cells that expressed rat hydroxysteroid sulfotransferase, .alpha.-hydroxytamoxifen was mutagenic and formed the same pattern of DNA adducts as that found in the liver of tamoxifen-treated rats. .alpha.-Hydroxytamoxifen was not activated, or was at least 20 times less active in cells expressing human hydroxysteroid sulfotransferase. All the other six known human xenobiotic-metabolizing sulfotransferases were also expressed in *S. typhimurium*. None activated .alpha.-hydroxytamoxifen to a mutagen. These results suggest that the **risk** of DNA adduct formation, and cancer, in the human liver is low and explain why tamoxifen is a powerful carcinogen to the rat liver, and why std. short-term tests fail to detect its mutagenicity.

L118 ANSWER 2 OF 12 MEDLINE DUPLICATE 1

AB Understanding the factors which alter estrogen metabolism and activity in endometrial tissue is important because unopposed estrogen stimulation is an important **risk** factor in the development of endometrial carcinoma. The cyclic progression of the endometrium through proliferative and secretory phases is normally under the control of the ovarian hormones beta-estradiol (E2) and progesterone. One mechanism by which progesterone inhibits the activity of E2 in secretory endometrium is by elevating the degree of E2 sulfation, thereby reducing its ability to bind to the estrogen receptor and elicit a cellular response. Our laboratories have investigated the cytosolic **sulfotransferases** (STs) found in biopsies of both proliferative and secretory endometrium obtained from five normal pre-menopausal women who were not taking any drugs or steroids. Two of the human cytosolic STs were detected in human endometrial tissues. The phenol-sulfating form of phenol ST (P-**PST**) was found at varying levels in cytosol from both proliferative and secretory endometrium in all of the women studied but with no consistent correlation to the phase of the menstrual cycle. In contrast, estrogen ST (EST) was not detected in the proliferative endometrial cytosol of any of the women studied but was consistently found in all of the secretory endometrial cytosols. The presence and levels of these STs was confirmed by ST activity studies, immunoblot analysis and Northern blot analysis. These results indicate that the expression of EST in human endometrial tissues varies with the phase of the menstrual cycle and is most likely regulated by progesterone secreted from the ovaries.

L118 ANSWER 3 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Using reverse transcriptase-polymerase chain reaction amplification it was possible to detect the presence of type 1 human estrogen sulfotransferase (hEST1) mRNA in the **hormone-dependent** : MCF-7 and T-47D, and hormone-independent: MDA-MB-231 and MDA-MB-468, human breast cancer cells. The expression of this mRNA is significantly higher in the MDA-MB-468 cells and a correlation of this mRNA expression with the enzymatic activity was observed. The progestin promegestone (R-5020) at a low concentration (5×10^{-7} M) can significantly increase the estrogen sulfotransferase activity and its mRNA in the **hormone-dependent** MCF-7 and T-47D cells. As estrogen sulfates are biologically inactive, the stimulatory effect on sulfotransferase by promegestone may open attractive possibilities in the control of estradiol in human breast cancer. (C) 1998 Elsevier Science Ltd. All rights reserved.

L118 ANSWER 4 OF 12 MEDLINE DUPLICATE 2

AB It has become clear that several polymorphisms of human drug-metabolizing enzymes influence an individual's susceptibility for chemical carcinogenesis. This review gives an overview on relevant polymorphisms of four families of drug-metabolizing enzymes. Rapid acetylators (with respect to N-acetyltransferase NAT2) were shown to have an increased

risk of colon cancer, but a decreased **risk** of bladder cancer. In addition an association between a NAT1 variant allele (NAT*10, due to mutations in the polyadenylation site causing approximately two fold higher activity) and colorectal cancer among NAT2 rapid acetylators was observed, suggesting a possible interaction between NAT1 and NAT2. Glutathione S-transferases M1 and T1 (GSTM1 and GSTT1) are polymorphic due to large deletions in the structural gene. Meta-analysis of 12 case-control studies demonstrated a significant association between the homozygous deletion of GSTM1 (GSTM1-0) and lung cancer (odds ratio: 1.41; 95% CI: 1.23-1.61). Combination of GSTM1-0 with two allelic variants of cytochrome P4501A1 (CYP1A1), CYP1A1 m2/m2 and CYP1A1 Val/Val further increases the **risk** for lung cancer. Indirect mechanisms by which deletion of GSTM1 increases **risk** for lung cancer may include GSTM1-0 associated decreased expression of GST M3 and increased activity of CYP1A1 and 1A2. Combination of GST M1-0 and NAT2 slow acetylation was associated with markedly increased **risk** for lung cancer (odds ratio: 7.8; 95% CI: 1.4-78.7). In addition GSTM1-0 is clearly associated with bladder cancer and possibly also with colorectal, hepatocellular, gastric, esophageal (interaction with CYP1A1), head and neck as well as cutaneous cancer. In individuals with the GSTT1-0 genotype more chromosomal aberrations and sister chromatid exchanges (SCEs) were observed after exposure to 1,3-butadiene or various haloalkanes or haloalkenes. Evidence for an association between GSTT1-0 and myelodysplastic syndrome and acute lymphoblastic leukemia has been presented. A polymorphic site of GSTP1 (valine to isoleucine at codon 104) decreases activity to several carcinogenic diol epoxides and was associated with testicular, bladder and lung cancer. Microsomal epoxide hydrolase (mEH) is polymorphic due to amino acid variation at residues 113 and 139. Polymorphic variants of mEH were associated with hepatocellular cancer (His-113 allele), ovarian cancer (Tyr-113 allele) and chronic obstructive pulmonary disease (His-113 allele). Three human **sulfotransferases** (STs) are regulated by genetic polymorphisms (hDHEAST, hM-PST, TS PST). Since a large number of environmental mutagens are activated by STs an association with human cancer **risk** might be expected.

L118 ANSWER 5 OF 12 CANCERLIT

AB Metabolic pathways leading to carcinogen activation and inactivation appear to contribute to human cancer susceptibility. Phenolsulfotransferases represent an important family of enzymes for further study, since they are thought to mediate both bioactivation and detoxification reactions in experimental animals. The thermostable form of phenolsulfotransferase (TS-PST; ST1A3), which is polymorphic in humans, catalyzes the sulfation of important environmental carcinogens (e.g., N-hydroxy arylamines and N-hydroxy heterocyclic amines). However, its role in the development of specific endogenous human cancers is unknown. We have developed a simple and reproducible microtiter-plate colorimetric method for measurement of TS-PST activity, and have validated its use in a large epidemiological case-control study of colon cancer. Human platelet cytosols were used since platelet TS-PST activity was previously shown to correlate with human liver and colon TS-PST activity. Here we report that **sulfotransferase** (ST1A3, as measured by 2-naphthol activity) appears as a potential protective factor for colon cancer. The population (n=221) segregated into two distinct phenotypes based on TS-PST activity: 'fast' and 'slow'. However, the slow phenotype was more frequently associated with colon cancer than with controls (56% vs 40%, p=0.03), suggesting that TS-PST should be included in future models of cancer **risk**.

L118 ANSWER 6 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3

AB Gene-environment interaction is an important aspect of human cancer **risk**. Genetic polymorphisms in acetylation and N-oxidation have previously been described regarding their impact on the heterocyclic amine-induced **risk** for colon cancer. Here, we report that

another enzyme involved in the metabolism of food-borne carcinogens, **sulfotransferase** (ST1A3 measured by 2-naphthol activity), may function as a potential protective factor for colon cancer in humans. Initially characterized in human liver and colon (Chou et al., 1995), **TS-PST** activity can also be measured in platelets. A simple microtiter-based colorimetric technique was developed for use in this case-control study. African-Americans had a higher mean ST activity than Caucasians (2.32 +/- 0.24 versus 1.77 +/- 0.09 nmols/min per mg cytosolic protein, $P = 0.036$). Furthermore, the slow ST phenotype (ST less than or equal to 1.53) was more frequently associated with colon cancer than controls (57 versus 40%, $P = 0.026$). These data suggest that the ST1A3 isoform may play a role in the differential **risk** for colorectal cancer. (C) 1997 Elsevier Science B.V.

L118 ANSWER 7 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Human bronchial epithelial cells (BEC), a primary defense against inhaled materials, are the progenitor cells for bronchogenic carcinomas and have important metabolic capabilities. We used reverse transcriptase-polymerase chain reaction (RT-PCR) to identify xenobiotic metabolism enzymes expressed in primary BEC and alveolar macrophages (AM) of non-smoking volunteers. Cytochromes P450 (CYP) 1A1, 1B1, 2B7, 2E1, and 4B1 and microsomal epoxide hydrolase (mEH) were expressed in BEC but not AM. CYP2F1 was expressed in BEC, but it was expressed at barely detectable levels or not at all in AM. NADPH oxidoreductase (NADPH OR), microsomal glutathione transferase (GST 12), glutathione transferase mu, **phenol sulfotransferase (PST)**, thermolabile **phenol sulfotransferase (TL PST)**, and the clara cell-specific gene, CC10 were expressed in both BEC and AM. CYP3A4 and glucuronosyl transferases-1 and 2 were not expressed in either BEC or AM. In contrast to primary BEC, of the genes evaluated, the immortalized human bronchial epithelial cell line BEP2D constitutively expressed only CYP1A1, CYP2E1, NADPH OR, glucuronosyl transferase 1, GST 12, GST mu, **PST**, TL **PST**, and CC10. The loss of xenobiotic metabolism enzyme gene expression in the BEP2D cell line may result from either reduced exposure to inducing agents, or loss of differentiative characteristics in culture. It is clear from the data comparing BEC and AM that there are important intertissue differences in expression of xenobiotic metabolism enzymes.

L118 ANSWER 8 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

AB In addition to industrial sources, benzene is present in a component of cigarette smoke emissions. Toxicity of benzene most likely results from oxidative metabolism of benzene to reactive products. However, susceptibility to these toxic effects may be related to a balance between activation (phase I) and detoxication (phase II) reactions. In the present study, we have estimated kinetic parameters of the two major detoxication reactions for benzene metabolites-phenol sulfation and hydroquinone glucuronidation-in liver subcellular fractions from 10 humans, and single samples from mice and rats. The extent of oxidative metabolism of benzene by these liver samples has been reported previously. Here, initial rates of phenol sulfation varied 3-fold (range 0.309-0.919 nmol/mg protein/min) among human samples. Measured rates were faster in rats (1.195 nmol/mg protein/min) than in mice (0.458 nmol/mg protein/min). Initial rates of hydroquinone glucuronidation by human samples also varied 3-fold (range 0.101-0.281 nmol/mg protein/min). Hydroquinone glucuronidation was more rapid by mouse microsomes (0.218 nmol/mg protein/min) than by rat microsomes (0.077 nmol/mg protein/min). To integrate interindividual differences in various enzyme activities, a physiological compartmental model was developed that incorporates rates of both conjugation reactions and oxidation reactions. Model equations were solved for steady-state concentrations of phenol and hydroquinone attained in human, mouse and rat blood during continuous exposure to benzene (0.01 μ M in blood). Among the 10 human subjects, steady-state concentrations of phenol varied 6-fold (range 0.38-2.17 nM) and steady-state concentrations of hydroquinone

varied 5-fold (range 6.66-31.44 nM), Predicted steady-state concentrations of phenol were higher in mice compared with rats (2.28 and 0.83 nM respectively), Likewise, higher steady-state concentrations of hydroquinone were predicted in mice than in rats (42.44 and 17.99 nM respectively), Predicted steady-state concentrations of phenol and hydroquinone in mice were higher than predictions for the 10 human subjects, whereas predicted concentrations for rats fell among the human values. As such, our results underscore the importance of considering the balance between activation and detoxication reactions in the elimination of toxicants, Model simulations suggest that both phase I and phase II pathways influence the relative **risk** from exposure to benzene.

L118 ANSWER 9 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 4

AB Variations in the total capacity of the rat ovary to metabolize xenobiotics during different phases of the estrous cycle were studied. The level of the conjugating enzymes, phenol UDP-glucuronosyltransferase (pUDPGT; EC 2.4.1.17), **phenol sulfotransferase** (**pST**; EC 2.8.2.1) and glutathione transferases (EC 2.5.1.18) was determined in the ovary and compared with the corresponding hepatic activities. In addition, catalase (EC 1.11.1.6) and NAD(P)H: quinone oxidoreductase (EC 1.6.99.2) two other detoxifying enzymes, were assayed. In order to study the hormonal influences on detoxifying enzymes, mature rats were characterized with respect to their stage in the estrous cycle. Immature rats were treated with pregnant mare's serum gonadotropin (PMSG) for 2 or 3 days to enrich the ovaries in preovulatory follicles or corpora lutea, respectively. The present study demonstrates that ovarian pUDPGT and **pST** activities are increased 936% and 175%, respectively, in ovaries enriched in corpora lutea compared to ovaries from untreated immature rats. Increases in these activities in mature rats during the metestrous stage of the estrous cycle compared to the proestrous stage were also noted. In the liver pUDPGT activity is increased significantly (1.6-fold) in immature rats with ovaries enriched in preovulatory follicles compared to untreated rats. Both ovarian **pST** and pUDPGT activities increased in mature rats treated with PMSG ('hyperstimulated'), while in the liver only **pST** was increased by such treatment. Ovarian glutathione transferase activity proved not to be dependent on the hormonal fluctuations associated with the estrous cycle. However, in the liver of mature rats treated with PMSG, this activity increased a-fold compared to the untreated immature rats. The catalase activity found in the ovarian mitochondrial fraction was approx. 10-fold higher than in the cytosolic fraction, independent of the hormonal status. Moreover, we found a significant 1.4-fold increase in peroxisomal catalase activity in the mitochondrial fraction of immature rats treated with PMSG, both when enriched in preovulatory follicles and in corpora lutea. In the liver cytosolic catalase activity decreased several-fold in immature rats following PMSG treatment. We did not find any variations in ovarian NAD(P)H:quinone oxidoreductase activity during the estrous cycle, whereas in the liver this activity decreased in the luteal phase, as it did in mature rats treated with PMSG. From this study and earlier investigations in our laboratory, we conclude that cyclic variations due to hormones of the estrous cycle of the major 7,12-dimethylbenz(a)anthracene (DMBA)-metabolizing phase I enzymes in the ovary are not accompanied by increases in the activities of the corresponding phase II enzymes. An increased steady-state level of reactive intermediates around the time of ovulation may thus increase the **risk** of cellular damage, e.g. in the oocyte, during this period.

L118 ANSWER 10 OF 12 LIFESCI COPYRIGHT 2002 CSA

AB Variations in the total capacity of the rat ovary to metabolize xenobiotics during different phases of the estrous cycle were studied. The level of the conjugating enzymes, phenol UDP-glucuronosyltransferase (pUDPGT; EC 2.4.1.17), **phenol sulfotransferase** (**pST**; EC 2.8.2.1) and glutathione transferases (EC 2.5.1.18) was determined in the ovary and compared with the corresponding hepatic

activities. In addition, catalase (EC 1.11.1.6) and NAD(P)H: quinone oxidoreductase (EC 1.6.99.2) two other detoxifying enzymes, were assayed. In order to study the hormonal influences on detoxifying enzymes, mature rats were characterized with respect to their stage in the estrous cycle. Immature rats were treated with pregnant mare's serum gonadotropin (PMSG) for 2 or 3 days to enrich the ovaries in preovulatory follicles or corpora lutea, respectively. The present study demonstrates that ovarian pUDPGT and pST activities are increased 936% and 175%, respectively, in ovaries enriched in corpora lutea compared to ovaries from untreated immature rats. Increases in these activities in mature rats during the metestrous stage of the estrous cycle compared to the proestrous stage were also noted. In the liver pUDPGT activity is increased significantly (1.6-fold) in immature rats with ovaries enriched in preovulatory follicles compared to untreated rats. Both ovarian pST and pUDPGT activities increased in mature rats treated with PMSG ("hyperstimulated"), while in the liver only pST was increased by such treatment. Ovarian glutathione transferase activity proved not to be dependent on the hormonal fluctuations associated with the estrous cycle. However, in the liver of mature rats treated with PMSG, this activity increased 2-fold compared to the untreated immature rats. The catalase activity found in the ovarian mitochondrial fraction was approx. 10-fold higher than in the cytosolic fraction, independent of the hormonal status. Moreover, we found a significant 1.4-fold increase in peroxisomal catalase activity in the mitochondrial fraction of immature rats treated with PMSG, both when enriched in preovulatory follicles and in corpora lutea. In the liver cytosolic catalase activity decreased several-fold in immature rats following PMSG treatment. We did not find any variations in ovarian NAD(P)H: quinone oxidoreductase activity during the estrous cycle, whereas in the liver this activity decreased in the luteal phase, as it did in mature rats treated with PMSG. From this study and earlier investigations in our laboratory, we conclude that cyclic variations due to hormones of the estrous cycle of the major 7,12-dimethylbenz(a)anthracene (DMBA)-metabolizing phase I enzymes in the ovary are not accompanied by increases in the activities of the corresponding phase II enzymes. An increased steady-state level of reactive intermediates around the time of ovulation may thus increase the **risk** of cellular damage, e.g. in the oocyte, during this period.

L118 ANSWER 11 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

AB 1. Methyl conjugation is an important pathway in the biotransformation of many drugs and xenobiotic compounds. 'Pharmacogenetic' variation exists in the activities of many methyltransferase enzymes, and experiments with the drug-metabolizing enzyme thiopurine methyltransferase (TPMT) offer a model for one approach that has proven useful in the study of methyltransferase pharmacogenetics.

2. TPMT catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine. This enzyme activity is present in the human red blood cell (RBC), and RBC TPMT activity is controlled by a common genetic polymorphism that regulates also the enzyme activity in all other human tissues that have been studied.

3. Subjects with inherited low levels of TPMT activity are at increased **risk** for thiopurine drug-induced myelotoxicity, while patients with high TPMT activities may be 'undertreated' with these drugs.

4. TPMT activity in tissue from selected strains of inbred mice also is regulated by a genetic polymorphism. These mice provide an animal model for use in the study of pharmacological or toxicological consequences of inherited differences in TPMT activity.

4. Other methyltransferase enzymes including thiol methyltransferase, catechol O-methyltransferase, and histamine N-methyltransferase also are present in the human RBC, are regulated by inheritance, and are responsible for individual variation in drug metabolism. Enhanced understanding of the pharmacogenetics of methylation may make it possible to understand and predict individual variation in the biotransformation, toxicity and therapeutic effect of compounds that undergo methyl

conjugation.

L118 ANSWER 12 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)

AB The metabolic pathways associated with carcinogenic aromatic amines in humans provide an excellent example of polymorphisms that appear to be relevant to human carcinogenesis. In this regard, the N-acetylation of arylamines and the O-acetylation of their N-hydroxy metabolites are catalyzed preferentially by a genetically polymorphic acetyltransferase, high activity of which has been correlated with decreased **risk** for urinary bladder cancer and increased susceptibility to colorectal cancer. Cytochrome P450IA2, the principal liver enzyme involved in aromatic amine N-oxidation, exhibits a wide interindividual variation that appears trimodal in several populations and is clearly inducible by cigarette smoking and probably other host factors as well. UDP-Glucuronosyltransferases, which catalyze the N-glucuronidation of N-hydroxyarylamines and are likely to be responsible for their transport to the colon, show widely varied but unimodal distributions in humans. In contrast, human liver sulfotransferase activity for N-hydroxyarylamines, which would be expected to decrease their transport through the circulation, is catalyzed by a polymorphic enzyme(s) that is expressed at higher levels in blacks, as compared to whites, and could contribute to their relatively lower incidence of urinary bladder cancer. Peroxidative activation of aromatic amines can also occur, especially from prostaglandin H synthase in the urinary bladder and myeloperoxidase in the lungs of cigarette smokers, and both show considerable individual variability, apparently due to the extent of tissue inflammation. In a pilot study, we have examined two of these polymorphisms, acetyltransferase and cytochrome P450IA2, in colorectal cancer/polyp cases (n = 38) and controls (n = 100) and found that, individuals who are both rapid acetylators and rapid N-oxidizers are indeed more prevalent (p < 0.008) among cases (37%) than among controls (16%).

=> s 152 and cancer

FILE 'MEDLINE'

388880 CANCER

L119 5 L40 AND CANCER

FILE 'SCISEARCH'

395418 CANCER

L120 33 L41 AND CANCER

FILE 'LIFESCI'

41125 CANCER

L121 2 L42 AND CANCER

FILE 'BIOTECHDS'

15868 CANCER

L122 1 L43 AND CANCER

FILE 'BIOSIS'

408395 CANCER

L123 12 L44 AND CANCER

FILE 'EMBASE'

637824 CANCER

L124 9 L45 AND CANCER

FILE 'HCAPLUS'

170275 CANCER

L125 19 L46 AND CANCER

FILE 'NTIS'

13106 CANCER

L126 0 L47 AND CANCER
 FILE 'ESBIOBASE'
 215126 CANCER
 L127 11 L48 AND CANCER
 FILE 'BIOTECHNO'
 104244 CANCER
 L128 5 L49 AND CANCER
 FILE 'WPIDS'
 34366 CANCER
 L129 0 L50 AND CANCER
 FILE 'CANCERLIT'
 387550 CANCER
 L130 6 L51 AND CANCER
 TOTAL FOR ALL FILES
 L131 103 L52 AND CANCER
 => s l131 not 1999-2002/PY
 FILE 'MEDLINE'
 1798929 1999-2002/PY
 L132 3 L119 NOT 1999-2002/PY
 FILE 'SCISEARCH'
 3578296 1999-2002/PY
 L133 16 L120 NOT 1999-2002/PY
 FILE 'LIFESCI'
 360388 1999-2002/PY
 L134 1 L121 NOT 1999-2002/PY
 FILE 'BIOTECHDS'
 53832 1999-2002/PY
 L135 0 L122 NOT 1999-2002/PY
 FILE 'BIOSIS'
 1949185 1999-2002/PY
 L136 5 L123 NOT 1999-2002/PY
 FILE 'EMBASE'
 1596024 1999-2002/PY
 L137 6 L124 NOT 1999-2002/PY
 FILE 'HCAPLUS'
 3421986 1999-2002/PY
 L138 5 L125 NOT 1999-2002/PY
 FILE 'NTIS'
 62667 1999-2002/PY
 L139 0 L126 NOT 1999-2002/PY
 FILE 'ESBIOBASE'
 1032395 1999-2002/PY
 L140 8 L127 NOT 1999-2002/PY
 FILE 'BIOTECHNO'
 434498 1999-2002/PY
 L141 4 L128 NOT 1999-2002/PY
 FILE 'WPIDS'
 3010571 1999-2002/PY

L142 0 L129 NOT 1999-2002/PY

FILE 'CANCERLIT'

329161 1999-2002/PY

L143 4 L130 NOT 1999-2002/PY

TOTAL FOR ALL FILES

L144 52 L131 NOT 1999-2002/PY

=> dup rem l144

PROCESSING COMPLETED FOR L144

L145 27 DUP REM L144 (25 DUPLICATES REMOVED)

=> s l145 not l118

FILE 'MEDLINE'

L146 3 S L145

L147 2 S L118

L148 2 L146 NOT L147

FILE 'SCISEARCH'

L149 14 S L145

L150 7 S L118

L151 11 L149 NOT L150

FILE 'LIFESCI'

L152 0 S L145

L153 1 S L118

L154 0 L152 NOT L153

FILE 'BIOTECHDS'

L155 0 S L145

L156 0 S L118

L157 0 L155 NOT L156

FILE 'BIOSIS'

L158 2 S L145

L159 0 S L118

L160 2 L158 NOT L159

FILE 'EMBASE'

L161 2 S L145

L162 0 S L118

L163 2 L161 NOT L162

FILE 'HCAPLUS'

L164 1 S L145

L165 1 S L118

L166 0 L164 NOT L165

FILE 'NTIS'

L167 0 S L145

L168 0 S L118

L169 0 L167 NOT L168

FILE 'ESBIOBASE'

L170 4 S L145

L171 0 S L118

L172 4 L170 NOT L171

FILE 'BIOTECHNO'

L173 0 S L145

L174 0 S L118

L175 0 L173 NOT L174

FILE 'WPIDS'
L176 0 S L145
L177 0 S L118
L178 0 L176 NOT L177

FILE 'CANCERLIT'
L179 1 S L145
L180 1 S L118
L181 0 L179 NOT L180

TOTAL FOR ALL FILES
L182 21 L145 NOT L118

=> d tot

L182 ANSWER 1 OF 21 MEDLINE
TI Daidzein sulfoconjugates are potent inhibitors of sterol sulfatase (EC 3.1.6.2).
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1997 Apr 28) 233 (3) 579-83.
Journal code: 0372516. ISSN: 0006-291X.
AU Wong C K; Keung W M
AN 97312453 MEDLINE

L182 ANSWER 2 OF 21 MEDLINE
TI Expression of cytosolic **sulfotransferases** in normal mammary epithelial cells and breast **cancer** cell lines.
SO CANCER RESEARCH, (1996 Apr 1) 56 (7) 1551-5.
Journal code: 2984705R. ISSN: 0008-5472.
AU Falany J L; Falany C N
AN 96181304 MEDLINE

L182 ANSWER 3 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI Regulation of sulfotransferase mRNA expression in male and female rats of various ages
SO CHEMICO-BIOLOGICAL INTERACTIONS, (20 FEB 1998) Vol. 109, No. 1-3, pp. 299-313.
Publisher: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
ISSN: 0009-2797.
AU Klaassen C D (Reprint); Liu L; Dunn R T
AN 1998:275532 SCISEARCH

L182 ANSWER 4 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI Regulation of estrogen activity by sulfation in human MCF-7 breast **cancer** cells
SO ONCOLOGY RESEARCH, (APR 1997) Vol. 9, No. 11-12, pp. 589-596.
Publisher: COGNIZANT COMMUNICATION CORP, 3 HARTSDALE ROAD, ELMSFORD, NY 10523-3701.
ISSN: 0965-0407.
AU Falany J L; Falany C N (Reprint)
AN 1998:274909 SCISEARCH

L182 ANSWER 5 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI STEROID **SULFOTRANSFERASES**
SO JOURNAL OF ENDOCRINOLOGY, (SEP 1996) Vol. 150, Supp. S, pp. S87-S97.
ISSN: 0022-0795.
AU LUUTHE V (Reprint); BERNIER F; DUFORT I
AN 96:815424 SCISEARCH

L182 ANSWER 6 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI MOLECULAR-BIOLOGY OF STEROID SULFOTRANSFERASES
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1996) Vol. 784, pp. 137-148.
ISSN: 0077-8923.

AU LUUTHE V (Reprint); BERNIER F; DUFORT I; LABRIE F
AN 96:471679 SCISEARCH

L182 ANSWER 7 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI REGULATION OF ESTROGEN **SULFOTRANSFERASE** IN HUMAN ENDOMETRIAL
ADENOCARCINOMA CELLS BY PROGESTERONE
SO ENDOCRINOLOGY, (APR 1996) Vol. 137, No. 4, pp. 1395-1401.
ISSN: 0013-7227.
AU FALANY J L; FALANY C N (Reprint)
AN 96:261735 SCISEARCH

L182 ANSWER 8 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI HUMAN FETAL ADRENAL HYDROXYSTEROID SULFOTRANSFERASE - CDNA CLONING, STABLE
EXPRESSION IN V79 CELLS AND FUNCTIONAL-CHARACTERIZATION OF THE EXPRESSED
ENZYME
SO MOLECULAR AND CELLULAR ENDOCRINOLOGY, (JUL 1995) Vol. 112, No. 1, pp.
53-60.
ISSN: 0303-7207.
AU FORBES K J; HAGEN M; GLATT H; HUME R; COUGHTRIE M W H (Reprint)
AN 95:569030 SCISEARCH

L182 ANSWER 9 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI SULFATION OF ENDOGENOUS COMPOUNDS AND XENOBIOTICS - INTERACTIONS AND
FUNCTION IN HEALTH AND DISEASE
SO CHEMICO-BIOLOGICAL INTERACTIONS, (JUN 1994) Vol. 92, No. 1-3, pp. 247-256.
ISSN: 0009-2797.
AU COUGHTRIE M W H (Reprint); BAMFORTH K J; SHARP S; JONES A L; BORTHWICK E
B; BARKER E V; ROBERTS R C; HUME R; BURCHELL A
AN 94:528725 SCISEARCH

L182 ANSWER 10 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI STEROID SULFATION BY EXPRESSED HUMAN CYTOSOLIC **SULFOTRANSFERASES**
SO JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (MAR 1994) Vol. 48,
No. 4, pp. 369-375.
ISSN: 0960-0760.
AU FALANY C N (Reprint); WHEELER J; OH T S; FALANY J L
AN 94:213239 SCISEARCH

L182 ANSWER 11 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI CLONING AND EXPRESSION OF CDNA-ENCODING HUMAN PLACENTAL ESTROGEN
SULFOTRANSFERASE
SO MOLECULAR AND CELLULAR ENDOCRINOLOGY, (FEB 1994) Vol. 99, No. 1, pp.
R11-R15.
ISSN: 0303-7207.
AU BERNIER F; SOLACHE I L; LABRIE F; LUUTHE V (Reprint)
AN 94:200864 SCISEARCH

L182 ANSWER 12 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI IDENTIFICATION AND CHARACTERIZATION OF CYTOSOLIC **SULFOTRANSFERASE**
ACTIVITIES IN MCF-7 HUMAN BREAST-CARCINOMA CELLS
SO JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (OCT 1993) Vol. 46,
No. 4, pp. 481-487.
ISSN: 0960-0760.
AU FALANY J L; LAWING L; FALANY C N (Reprint)
AN 93:665393 SCISEARCH

L182 ANSWER 13 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)
TI CHARACTERIZATION OF ESTRONE SULFATASE ACTIVITY IN HUMAN THROMBOCYTES
SO JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (AUG 1993) Vol. 46,
No. 2, pp. 215-226.
ISSN: 0960-0760.
AU SOLIMAN H R (Reprint); DIRE D; BOUDOU P; JULIEN R; LAUNAY J M; BRERAULT J
L; VILLETTE J M; FIET J
AN 93:532827 SCISEARCH

- L182 ANSWER 14 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI Studies on the effect of schistosomal infection and dimethyl nitrosamine on liver detoxifying enzymes of male mice.
 SO Journal of the Medical Research Institute, (1994) Vol. 15, No. 2, pp. 129-141.
 AU Ebied, Samia (1); El-Sharkawy, Awatef (1); El-Shazly, Laila; Mina, B.; El-Sewedy, S. (1); Bacha, Laila Abou; Ibrahim, Amany (1); Abdelsalam, A.
 AN 1995:350010 BIOSIS
- L182 ANSWER 15 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI PLATELET MONO AMINE OXIDASE AND PHENOL SULFO TRANSFERASE EC-2.8.2.1 M FORM AND P FORM IN **CANCER**.
 SO CLIN CHIM ACTA, (1984) 139 (3), 303-312.
 CODEN: CCATAR. ISSN: 0009-8981.
 AU RAMPLING R P; CARTER S M B; GLOVER V; SANDLER M
 AN 1984:332090 BIOSIS
- L182 ANSWER 16 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
 TI Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase: Potential role in drug metabolism and chemoprevention.
 SO Drug Metabolism and Disposition, (1996) 24/2 (232-237).
 ISSN: 0090-9556 CODEN: DMDSAI
 AU Eaton E.A.; Walle U.K.; Lewis A.J.; Hudson T.; Wilson A.A.; Walle T.
 AN 96061847 EMBASE
- L182 ANSWER 17 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
 TI Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by human **sulfotransferase(s)**.
 SO Cancer Research, (1995) 55/3 (525-529).
 ISSN: 0008-5472 CODEN: CNREA8
 AU Chou H.-C.; Lang N.P.; Kadlubar F.F.
 AN 95040333 EMBASE
- L182 ANSWER 18 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AN 1998189253 ESBIOWASE
 TI Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz .alpha. anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase
 AU Chou H.-C.; Ozawa S.; Fu P.P.; Lang N.P.; Kadlubar F.F.
 CS F.F. Kadlubar, Natl Ctr Toxicological Res (HFT-100), Jefferson, AR 72079, United States.
 SO Carcinogenesis, (1998), 19/6 (1071-1076), 60 reference(s)
 CODEN: CRNGDP ISSN: 0143-3334
 DT Journal; Article
 CY United Kingdom
 LA English
 SL English
- L182 ANSWER 19 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AN 1995053533 ESBIOWASE
 TI Formation of 2-amino-3-methylimidazo 4,5-f quinoline- and 2-amino-3,8-dimethylimidazo 4,5-f quinoxaline-sulfamates by cDNA-expressed mammalian **phenol sulfotransferases**
 AU Ozawa S.; Nagata K.; Yamazoe Y.; Kato R.
 CS S. Ozawa, Japan.
 SO Japanese Journal of Cancer Research, (1995), 86/3 (264-269)
 CODEN: JJCREP ISSN: 0910-5050
 DT Journal; Article
 LA English
- L182 ANSWER 20 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AN 1995042276 ESBIOWASE
 TI Metabolic activation of the N-hydroxy derivative of the carcinogen

4-aminobiphenyl by human tissue **sulfotransferases**
 AU Chou H.-C.; Lang N.P.; Kadlubar F.F.
 CS H.-C. Chou, Office of Research (NFT-100), National Center Toxicological
 Res., Jefferson, AR 72079, United States.
 SO Carcinogenesis, (1995), 16/2 (413-417)
 CODEN: CRNGDP ISSN: 0143-3334
 DT Journal; Article
 CY United Kingdom
 LA English
 SL English

L182 ANSWER 21 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AN 1995011446 ESBIOBASE
 TI Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo 4,5-b pyridine by
 cDNA-expressed human and rat arylsulfotransferases
 AU Ozawa S.; Chou H.-C.; Kadlubar F.F.; Nagata K.; Yamazoe Y.; Kato R.
 CS S. Ozawa, Department of Pharmacology, School of Medicine, Keio
 University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.
 SO Japanese Journal of Cancer Research, (1994), 85/12 (1220-1228)
 CODEN: JJCREP ISSN: 0910-5050
 DT Journal; Article
 CY Japan
 LA English
 SL English

=> d ab tot

L182 ANSWER 1 OF 21 MEDLINE
 AB Recent studies have associated high dietary isoflavone intake with low
 incidence of breast **cancer**. Since estrogenic steroids are
 important factors in the evolution of breast **cancer**, and in
 breast tumors they are derived mainly from the sterol sulfatase pathway,
 we have therefore investigated effects of the isoflavone daidzein and its
 sulfoconjugates, daidzein-4'-O-sulfate and daidzein-7,4'-di-O-sulfate, on
 sterol sulfatase activity using dehydroepiandrosterone sulfate as
 substrate. While daidzein does not affect sterol sulfatase, its
 sulfoconjugates are potent inhibitors of this enzyme. Kinetic analyses
 reveal that daidzein-4'-O-sulfate and daidzein-7,4'-di-O-sulfate inhibit
 sterol sulfatase competitively with respect to the steroid substrate and
 with Ki values of 5.9 and 1 microM, respectively. Daidzein
 sulfo-conjugates also inhibit hydroxysteroid and **phenol**
sulfotransferases but at much higher concentrations. These results
 provide a biochemical basis for the putative chemopreventive role of
 dietary isoflavones against breast **cancer**.

L182 ANSWER 2 OF 21 MEDLINE
 AB Breast cancers require the presence of estrogens for the maintenance of
 growth at some time in the course of their development, as does normal
 breast tissue. Sulfation is an important process in the metabolism and
 inactivation of steroids, including estrogens, because the addition of the
 charged sulfonate group prevents the binding of the steroid to its
 receptor. Also, many of the therapeutic and chemopreventive agents used in
 the treatment of breast **cancer** are substrates for the
sulfotransferases (STs). The activity and expression of four
 cytosolic STs, which are the human phenol-sulfating and
 monoamine-sulfating forms of phenol ST (**PST**),
 dehydroepiandrosterone ST, and estrogen ST (hEST), were assayed in normal
 breast cells and in breast **cancer** cell lines. ST activities and
 immunoreactivities were assayed in the estrogen receptor-positive human
 breast **cancer** cell lines ZR-75-1, T-47D and MCF-7; in the
 estrogen receptor-negative breast **cancer** cell lines BT-20,
 MDA-MB-468, and MDA-MB-231; and in normal human mammary epithelial cells.
 The PSTs were the most highly expressed ST activities in the breast

cancer cell lines, although the levels of activity varied significantly. ZR-75-1 and BT-20 cells possessed the highest levels of activity of the human phenol-sulfating form of **PST**. The breast **cancer** cell lines showed only trace levels of dehydroepiandrosterone ST and hEST activities. In contrast, hEST was the only ST detectable in human mammary epithelial cells. Understanding the regulation of ST activity in these breast **cancer** and normal breast cell lines will improve our knowledge of the role of sulfation in breast **cancer** and provide a model with which to study the mechanism of action of estrogens in mammary cells.

L182 ANSWER 3 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Sulfotransferases (SULTs) are Phase II drug-metabolizing enzymes that catalyze the addition of a sulfuryl moiety to both endogenous compounds, including steroids and neurotransmitters, and certain xenobiotics, including N-hydroxy-2-acetylaminofluorene and phenolic compounds, like a-naphthol. In contrast to certain Phase I drug-metabolizing enzymes, little is known about the regulation of the sulfotransferases. These series of studies were designed to analyze SULT mRNA expression and hormonal regulation in male and female rats. The hepatic expression of six different SULT isoforms was examined including three phenol SULTs and three hydroxysteroid SULTs. SULT mRNA expression was examined in adult and developing rats, as well as, in hypophysectomized (HX) and growth hormone-supplemented HX animals. SULT1A1 is thought to be important for the sulfation of simple phenols and its mRNA expression is about twice as high in adult male as in female rats. This difference in SULT1A1 mRNA levels is largely due to a greater decrease in mRNA levels after puberty in female than in male rats. Hypophysectomy resulted in a decrease in expression of SULT1A1 mRNA in both male and female rats. Replacement of growth hormone (GH) by either intermittent injection (male pattern) or infusion (female pattern) failed to restore SULT1A1 expression. Sulfotransferase SULT1C1 has been implicated in activation of N-hydroxyacetylaminofluorene. In contrast to SULT1A1, SULT1C1 mRNA expression is about 10-fold higher in adult males than in adult female rats. This male-dominant expression pattern emerges at 40-50 days of age and is due to an increase in SULT1C1 mRNA in males. Hypophysectomy abolished SULT1C1 expression in male rats. Interestingly, replacement of GH by injection (male pattern) restored SULT1C1 mRNA expression in males and enhanced SULT1C1 expression in female rats to levels observed in adult male rats.

GH infusion (female pattern) did not affect SULT1C1 mRNA expression in either male or female rats. Estrogen sulfotransferase (SULT1E2) may play a role in estrogen homeostasis. Adult male rats express SULT1E2 mRNA at levels 10-fold higher than those observed in adult females and similar to SULT1C1, this is due to an increase in SULT1E2, mRNA occurring during puberty in the male rat. Hypophysectomy did not appreciably affect SULT1E2 expression in male rats; however in contrast to males, hypophysectomy markedly enhanced SULT1E2 expression in female rats. GH infusion suppressed SULT1E2 levels in HX male rats. The expression of hydroxysteroid sulfotransferases was also examined. The SULT-20/21 isoform was expressed in both male and female rats. Male expression of this isoform peaked at 30 days of age and then declined to similar to 30% of the level observed in adult females. SULT-20/21 mRNA expression increased sharply at 45 days of age in female rats and remained elevated. Expression of SULT-20/21 mRNA was decreased markedly by hypophysectomy in both male and female rats. GH injection did not affect SULT-20/21 mRNA expression in HX males, however this treatment resulted in a 4-fold increase in SULT-20/21 mRNA in HX females. GH infusion restored SULT-20/21 expression in HX-male rats. GH infusion did elevate SULT-20/21 mRNA expression in female-HX rats, but not to the level observed in intact females. Hydroxysteroid SULT isoform SULT-40/41 was expressed in adult female but not adult male rats, SULT-40/41 expression peaked at 15 days of age in both male and female rats and decreased thereafter. The decrease in expression was more pronounced in male rats. SULT-60 mRNA, like

SULT-40/41, was expressed only in adult female rats. Male rats express SULT-60 at 30 days of age, but SULT-60 mRNA is undetectable at 60 days. SULT-60 mRNA was expressed in females only after day 30 and female SULT-60 mRNA expression remains high thereafter. SULT-40/41 and SULT-60 mRNA expression was increased by HX in male rats and decreased by HX in female rats. GH injection suppressed the expression of SULT-40/41 and SULT-60 in males, but did not alter their expression in females. GH infusion did not alter SULT-40/41 or SULT-60 expression in males, but slightly increased the expression of these isoforms in females. In summary, expression of **phenol sulfotransferase** SULT1A1 was found to be male predominant (2-fold greater) In contrast to isoforms SULT1C1 and SULT1E2, which were male-specific in that they were expressed at 10-fold higher levels in adult male than in female rats. SULT1A1 expression did not appear to be regulated by GH. SULT1C1 expression in males was controlled by the male GH secretory pattern, while in females SULT1E2, expression was suppressed by the female GH secretory pattern. The hydroxysteroid SULTs were primarily expressed in adult female rats, although transient expression in immature male rats was detected. SULT-20/21 was female predominant as it was expressed in adult male rats at 30% of the female level, whereas SULT-40/41 and SULT-60 were female specific as they were undetectable In adult male rats. GH infusion (female pattern) enhanced SULT-20/21 in HX rats, but had less of an effect on SULT-40/41 and SULT-60. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L182 ANSWER 4 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Estrogen metabolism is closely associated with the growth, progression, and treatment of breast **cancer** because many breast cancers are dependent upon estrogens for both growth and progression. Factors that affect the intracellular metabolism of estrogens may be critical in altering the effects of estrogens on breast **cancer** cells. MCF-7 cells have been used as a model system to study the effects of estrogens on breast **cancer** cellular growth. Because normal human mammary epithelial (HME) cells contain estrogen sulfotransferase (EST), which is involved in the inactivation of estrogens via sulfation, and MCF-7 cells do not possess this enzyme, the absence of EST may be critical to the growth of MCF-7 cells in the presence of estrogens. To study the effects of EST on cellular growth, MCF-7 cells stably transformed with an EST expression vector were compared to control cells transformed with vector only. Sulfation of 20 nM E-2 occurs more rapidly with MCF-7 cells transformed with EST than with the control cells, thereby rendering E-2 physiologically inactive. Additionally, these EST/MCF-7 cells sulfate 20 nM 17 alpha-ethinylestradiol (EE2) at a rate similar to that for E-2 but sulfate 20 nM diethylstilbestrol (DES) much more slowly; these results correlate with the kinetic characteristics of EST for these steroids. EST/MCF-7 cells require higher concentrations of E-2 to stimulate growth than do control MCF-7 cells, hypothetically because EST is inactivating E-2 via sulfation, rendering it incapable of binding to the estrogen receptor (ER). The effects of EE2 are similar to those of E-2 whereas DES is effective at lower concentrations because it is not inactivated by EST. Neither control nor EST/MCF-7 cells grow well in the complete absence of estrogens, as would be expected because MCF-7 cells are estrogen dependent. However, in medium that has not been treated to remove endogenous estrogens, EST/MCF-7 cells grow more slowly than control cells, most likely because EST is inactivating the estrogens in the medium, making them ineffective in stimulating growth. EST/MCF-7 cells possess EST at levels similar to HME cells and are less responsive to estrogens than are MCF-7 cells lacking EST. The loss of EST may be a factor in oncogenesis, which leads to altered estrogen metabolism in breast carcinoma cells.

L182 ANSWER 5 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Human dehydroepiandrosterone **sulfotransferase** (DHEA-ST) catalyzes the sulfonation of DHEA, cholesterol, pregnenolone as well as androsterone. RNA blot analysis shows two DHEA-ST mRNA species of 1.3 and

1.8 kb that are expressed similarly in liver and adrenals. To determine whether the form expressed in adrenals is distinct or identical with the one expressed in liver, we have cloned and sequenced the 1.8 kb DHEA-ST cDNA from human adrenal cDNA library. Except for one nucleotide difference, the human adrenal and liver DHEA-ST cDNAs are identical. Using expression vectors containing the chloramphenicol acetyltransferase (CAT) reporter gene ligated to various fragments of the DHEA-ST gene promoter, we have shown that DHEA-ST gene promoter activity is stimulated by estradiol (E(2)). The E(2) stimulation is inhibited by the anti-estrogen EM-139. In contrast to human DHEA-ST, guinea pig hydroxysteroid **sulfotransferases** show high substrate- and stereo-selectivity. We have cloned a chiral-specific pregnenolone **sulfotransferase** (PREG-ST) which catalyzes mainly the transformation of pregnenolone to pregnenolone sulfate.

Estrogen **sulfotransferase** catalyzes the conversion of estrone and estradiol to their inactive sulfated forms and could thus play a major role in the control of estrogen levels in target tissues. Recently, using a probe derived from bovine estrogen **sulfotransferase**, we have cloned a cDNA and gene that we first named human estrogen **sulfotransferase** (hEST) since the expressed enzyme is able to transform estrone to estrone sulfate. Actually, the Hugo nomenclature committee named this gene STM gene because it also codes for monoamine-sulfating phenolsulfotransferase (M-PST). hEST1 possesses the same coding and 3'-untranslated region as human brain aryl **sulfotransferase** (HAST) and M-PST, but different 5'-noncoding region. Analysis of hEST1 gene sequence indicates that hEST1 and HAST3 or M-PST mRNA species are transcribed from a single hEST1 gene by alternative promoters using two separate exon I, named exon Ia and exon Ib. We also described the identification of a third mRNA species (M-PST gamma) issued from the STM gene and the characterization of the structure of the phenol-sulfating phenolsulfotransferase (STP) gene that is highly homologous to the STM gene. Similar to STM, the STP gene generates multiple mRNA species that differ only in the 5'-untranslated sequence.

L182 ANSWER 6 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

L182 ANSWER 7 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB During the secretory phase of the human menstrual cycle, the endometrium is minimally responsive to the estrogens secreted from the ovaries. Conjugation of beta-estradiol (E(2)) with sulfate is thought to be an important mechanism in the regulation of the levels of active E(2) in endometrial tissue. Estrogen sulfation is reportedly increased during the secretory phase in response to the high levels of progesterone secreted by the ovaries. Estrogen **sulfotransferase** (hEST), a distinct form of human cytosolic **sulfotransferase** (ST) with an affinity for E(2) and estrone at low nanomolar concentrations, has recently been cloned and expressed in mammalian cells and in bacteria (J Steroid Biochem Mol Biol 52:529, 1995). At least two other forms of human cytosolic ST, dehydroepiandrosterone ST (hDHEA-ST) and the phenol-sulfating form of phenol-ST (hP-PST), also conjugate estrogens but at micromolar concentrations. This report describes the specific induction of hEST in human Ishikawa endometrial adenocarcinoma cells by progesterone as a model for the increases in estrogen sulfation observed in women during the secretory phase of the menstrual cycle. Treatment of Ishikawa cells with 10 μ M progesterone for 48 h resulted in a 7-fold increase in the sulfation of 20 nM E(2). The sulfation of selective substrates for human dehydroepiandrosterone **sulfotransferase** (hDHEA-ST) and the two forms of **phenol sulfotransferase** (hP-PST, hM-PST) were not affected by treatment with progesterone. The levels of immunoreactive hEST and hEST mRNA in the Ishikawa cells were both increased by progesterone, whereas the levels of immunoreactive hDHEA-ST, hP-PST, and hM-PST were not altered. hEST activity was not induced by treatment

of Ishikawa cells with varying concentrations of E(2), testosterone, or cortisol. The induction of hEST by progesterone was inhibited by RU-486, indicating that progesterone is acting via the progesterone receptor. These results indicate that progesterone is capable of specifically inducing hEST and estrogen sulfation in human Ishikawa adenocarcinoma cells and suggest a mechanism for increasing estrogen sulfation in the endometrium during the secretory phase of the menstrual cycle.

L182 ANSWER 8 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Dehydroepiandrosterone sulphate (DHEAS) is a major adrenal secretory product, particularly in the fetus where it serves as a substrate for oestrogen biosynthesis by the placenta. The enzyme in the adrenal responsible for synthesising DHEAS, hydroxysteroid sulphotransferase (HST), is therefore essential for human development. We have isolated a full-length cDNA clone, encoding human fetal adrenal HST, and constructed a stable cell line expressing it by transfection into V79 Chinese hamster lung fibroblast cells. This cDNA was essentially identical to that isolated from adult human liver, where the role of HST is less well understood. This recombinant cell line allowed determination of the substrate specificity and kinetic properties of this enzyme towards various steroid hormones, and by comparison of these activities with human liver cytosol we have shown that HST is the major sulphotransferase responsible for the sulphation of DHEA, androsterone and pregnenolone in man and that, functionally, the hepatic and adrenal enzymes are very similar. The expressed HST was also active with testosterone, cortisol (although at low levels) and the xenobiotic 17 alpha-ethinyloestradiol, but not with oestrone or 1-naphthol. We have therefore created a valuable resource for the study of this important enzyme.

L182 ANSWER 9 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Sulfation is a major detoxication mechanism for endogenous compounds and xenobiotics performed by a family of sulfotransferase isoenzymes. Understanding the normal cellular functions of these different sulfotransferases and the way in which endogenous and exogenous factors are able to influence their activity and expression will provide us with the information necessary to develop novel therapeutic strategies for conditions where sulfation may be implicated. This concept is discussed and is illustrated by examples including adverse drug reactions, fetal development and **cancer**.

L182 ANSWER 10 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB The human cytosolic **sulfotransferases** (STs), dehydroepiandrosterone **sulfotransferase** (DHEA-ST) and the **phenol-sulfating** form of **phenol sulfotransferase**, (P-**PST**), have been expressed in bacteria and used to investigate the ability of the cloned enzymes to conjugate steroids and related compounds. DHEA-ST was capable of sulfating all of the 3-hydroxysteroids, testosterone and estrogens tested as substrates. The 3-hydroxysteroids, androsterone, epiandrosterone and androstenediol, were conjugated at 50-60% of the rate of DHEA. Of the steroids tested, P-**PST** was capable of conjugating only the estrogens. The catechol estrogens, 2-hydroxyestradiol, 4-hydroxyestradiol and 4-hydroxyestrone, and compounds with estrogenic activity such as 17 alpha-ethinyloestradiol and trans-4-hydroxytamoxifen, were also tested as substrates. DHEA-ST showed little or no sulfation activity with these compounds; however, all of these compounds were sulfated by P-**PST**. These results indicate that the expressed human STs are valuable in analyzing the overlapping substrate specificities of these enzymes and that P-**PST** may have an important role in the metabolism of estrogens and estrogenic compounds in human tissues.

L182 ANSWER 11 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Using two oligoprimers derived from the bovine placental estrogen sulfotransferase sequence, we amplified a probe for human placental

estrogen sulfotransferase. Using this probe to screen a human placental cDNA library constructed in lambda gt11, we isolated a cDNA clone of 1.3 kb encoding human estrogen sulfotransferase. DNA analysis predicts a protein of 295 amino acids with a calculated molecular weight of 34199. Alignment of the amino acid sequence with other sulfotransferases indicates that human placental estrogen sulfotransferase shares 68.6, 68.2 and 65.9% similarity with bovine placental, guinea pig adrenocortical, and rat liver estrogen sulfotransferase, respectively. It shows also 95.6, 57.6, 85.3, and 54.2% similarity to human phenol, human DHEA, rat **phenol**, and rat hydroxysteroid **sulfotransferase**, respectively. Transfection of expression vectors encoding human estrogen sulfotransferase and dehydroepiandrosterone (DHEA) sulfotransferase in human adrenal adenocarcinoma SW-13 cells indicates that estrogen sulfotransferase transforms estrone more specifically, whereas DHEA sulfotransferase is more specific for DHEA and pregnenolone.

L182 ANSWER 12 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB MCF-7 human mammary carcinoma cells have been reported to possess beta-estradiol and dehydroepiandrosterone **sulfotransferase** activities. These steroid **sulfotransferase** activities may be important in the metabolism and activity of different steroids in these cells. This report describes and characterizes both the enzymatic activity of three cytosolic **sulfotransferases** found in MCF-7 cells and the corresponding immunoblot analysis of these enzymes with specific anti-**sulfotransferase** antibodies. Two cytosolic **sulfotransferases** have been purified and characterized from human tissues which are capable of sulfating estrogens. These are the **phenol**-sulfating form of **phenol sulfotransferase** (P-PST) and the hydroxysteroid **sulfotransferase**, dehydroepiandrosterone **sulfotransferase** (DHEA-ST). The results of this study show that P-PST is the major cytosolic **sulfotransferase** found in MCF-7 cytosol and is responsible for most of the fl-estradiol sulfation in these cells. Although DHEA-ST activity was found in MCF-7 cytosol, this activity was only about 3% of the P-PST activity. Immunoblot analysis of MCF-7 cytosol detected both P-PST and lower levels of the monoamine-sulfating form of **PST**; however DHEA-ST could not be detected apparently because of low levels of expression. Human liver P-PST was expressed in Cos-7 Green monkey kidney fibroblasts and the ability of the cloned enzyme to sulfate beta-estradiol was investigated. This study indicates that P-PST is the prevalent cytosolic **sulfotransferase** in MCF-7 cytosol and is responsible for the majority of fl-estradiol sulfation in these cells.

L182 ANSWER 13 OF 21 SCISEARCH COPYRIGHT 2002 ISI (R)

AB Estrone sulfatase is an important enzyme which catalyzes the production of estrone from estrone sulfate in a variety of human and animal tissues. We report, for the first time, on the presence of estrone sulfatase activity in thrombocytes from human blood. Incubation of [H-3]estrone sulfate in the presence of human thrombocyte lysates resulted in the formation of [H-3]estrone as assessed by two-dimensional TLC. Estrone sulfatase activity was localized in the mitochondrial-microsomal fraction in thrombocytes from human blood. The enzyme was thermostable and had an optimum pH of 5.60 in acetate buffer. The highest activity was obtained in the presence of 0.1% of either Nonidet P-40 or Triton X-100. Phosphate ions (1 mM) inhibited the enzyme activity by 64% and similar effects were observed in the presence of platelet-free plasma. Endogenous inhibitors had no effect on the observed enzyme activity under assay conditions as evidenced in this study. The apparent K(m) value was 3.16 +/- 0.08 muM for [H-3]estrone sulfate and V was 188.5 +/- 2.6 (mean +/- SEM, n = 22) pmol . mg protein-1.h-1. Comparison between two thrombocyte preparative procedures provided evidence that thrombocyte estrone sulfatase activity should be measured in thrombocyte samples representing the whole thrombocyte population. This parameter appeared critical for accurate

measurements of enzyme activity. The presence of estrone sulfatase activity in human thrombocytes provides a new non-invasive tool for the study of this activity both in physiological and pathological conditions which could be of potential clinical relevance.

L182 ANSWER 14 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

L182 ANSWER 15 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB **Phenol sulfotransferase** and monoamine oxidase inactivate a wide range of dietary and endogenous phenols/monoamines by sulfoconjugation and oxidative deamination, respectively. In this study, both enzymes were measured in platelets from **cancer** patients and controls. Of the 2 variants of **phenol sulfotransferase**, activity of the P form was normal in all groups. Activity of the M form was significantly less than control values in patients with **cancer** of the rectum and bowel, but not in other **cancer** patient groups. If this finding reflects enzyme activity elsewhere in the body and is not merely a manifestation of an abnormal platelet population, the deficit could expose affected subjects to the action of potentially carcinogenic dietary phenols. Platelet monoamine oxidase activity was significantly raised in the **cancer** group as a whole, and in all sub-types investigated apart from breast **cancer**. The increase in the **cancer** group as a whole was independent of sex, age, drugs, radiotherapy, smoking or platelet count. Its mechanism and significance are unknown but there may be links with the patients' psychiatric state.

L182 ANSWER 16 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

AB The common dietary constituent quercetin was a potent inhibitor of sulfoconjugation of acetaminophen and minoxidil by human liver cytosol, partially purified P-form phenolsulfotransferase (**PST**), and recombinant P-form **PST**, with IC50 values of 0.025-0.095 μ M. Quercetin inhibition of acetaminophen was noncompetitive with respect to acceptor substrate, with a K(i) value of 0.067 μ M. A number of other flavonoids, such as fisetin, galangin, myricetin, kaempferol, chrysin, and apigenin, were also potent inhibitors of P-form **PST**-mediated sulfation, with IC50 values < 1 μ M. Studies of structural analogs indicated the flavonoid 7-hydroxyl group as particularly important for potent inhibition. Potential human metabolites of quercetin were poor inhibitors. Curcumin, genistein, and ellagic acid (other polyphenolic natural products) were also inhibitors of P-form **PST**, with IC50 values of 0.38-34.8 μ M. Quercetin was also shown to inhibit sulfoconjugation by the human hepatoma cell line Hep G2. Although less potent in this intact cell system (IC50 2-5 μ M), quercetin was still more potent than 2,6-dichloro-4-nitrophenol, the classical P-form **PST** inhibitor that has been shown to be an inhibitor also in vivo. These observations suggest the potential for clinically important drug interactions, as well as a possible role for flavonoids as chemopreventive agents in sulfation-induced carcinogenesis.

L182 ANSWER 17 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

AB Several N-hydroxy metabolites of carcinogenic arylamines and heterocyclic amines were examined as substrates for bioactivation by human liver **sulfotransferases** (STs). Among the N-hydroxy derivatives studied, N-hydroxy-2-acetylaminofluorene, N-hydroxy-2-aminofluorene, N-hydroxy-4,4'-methylene-bis(2-chloroaniline), N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, and N-hydroxy-2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole were each metabolically activated by phosphoadenosine-5'-phosphosulfate-dependent human liver STs. No ST-mediated DNA binding of N-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline or N-hydroxy-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline was detected under our assay conditions. In the 12 human hepatic cytosols studied, the extent of 3'-phosphoadenosine-5'-phosphosulfate-dependent DNA binding of the N-hydroxy derivatives were all significantly correlated with levels of thermostable phenol ST (TS-**PST**) activity but not

with thermolabile phenol ST or dehydroepiandrosterone ST activities. The propensity of these N-hydroxy arylamines and N-hydroxy heterocyclic amines to serve as selective substrates for human TS-**PST** was further confirmed by inhibition with 2,6-dichloro-4-nitrophenol and by thermo-stability studies. N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,-5b]pyridine and N-hydroxy-4,4'-methylene-bis(2-chloroaniline) were also used as substrates to study ST-dependent metabolic activation in other human tissue preparations. 3'-phosphoadenosine-5'-phosphosulfate-dependent DNA binding activity was detected in human liver and colon cytosols but not in pancreas, larynx, or urinary bladder epithelial cytosols. Since the TS-**PST** appears to be expressed polymorphically in human populations, the finding that human TS-**PST** is capable of metabolically activating N-hydroxy metabolites of several carcinogenic arylamines and heterocyclic amines suggests that TS-**PST** may have an important role in determining interindividual susceptibility to these environmental and dietary carcinogens.

- L182 ANSWER 18 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AB Methyl-hydroxylated metabolites of the potent carcinogen, 7,12-dimethylbenz .alpha. anthracene (DMBA), namely, 7-hydroxymethyl-12-methylbenz .alpha. anthrac (7-OH-DMBA), 7-methyl-12-hydroxymethylbenz .alpha. anthrac (12-OH-DMBA) and 7,12-dihydroxymethylbenz .alpha. anthracene (7,12-diOH-DMBA), were examined as substrates for sulfotransferase bioactivation in different human tissue cytosols. Hepatic cytosols, which were able to catalyze the 3@
 ?-phosphoadenosine 5@?-phosphosulfate (PAPS)-dependent DNA binding of 7-OH-DMBA, 12-OH-DMBA and 7,12-diOH-DMBA, were highly sensitive to inhibition by dehydroepiandrosterone (DHEA), a specific substrate for human DHEA-steroid sulfotransferase (IC.sub.50 = 5 .mu.M). By comparison, 2,6-dichloro-4-nitrophenol, a potent inhibitor of the thermostable (TS)-phenol and estrogen sulfotransferases, did not have an appreciable inhibitory effect. Neither p-nitrophenol, a high affinity substrate for human TS-phenol and estrogen sulfotransferases, nor dopamine, a specific substrate for the thermolabile (TL)-phenol sulfotransferase, significantly inhibited the DNA binding of 12-OH-DMBA catalyzed by hepatic cytosols. Inter-subject variation (n = 12) of the PAPS-dependent DNA binding of 12-OH- and 7,12-diOH-DMBAs also correlated well with DHEA-sulfotransferase activity (r = 0.90; P < 0.00001 and r = 0.92; P < 0.00001, respectively). This sulfation-dependent metabolic activation was not detected in cytosols from human colon, pancreas, larynx or mammary gland. Both TS- and TL-phenol sulfotransferases were active in human liver and colon but only liver contained DHEA-sulfotransferase activity. These results indicate that the sulfotransferase-mediated activation of the methylhydroxylated DMBAs is predominantly catalyzed by DHEA-steroid sulfotransferase in human liver and that TS- and TL-phenol sulfotransferases and estrogen sulfotransferase are not involved in the catalysis.
- L182 ANSWER 19 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
- L182 ANSWER 20 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AB The role of human **sulfotransferase**(s) in the bioactivation of the N-hydroxy (N-OH) metabolite of the human bladder carcinogen 4-aminobiphenyl (ABP) was investigated in vitro with human tissue cytosols. Using an enzymatic assay consisting of a PAPS-regenerating system, .sup.3H N-OH-ABP, calf thymus DNA and tissue cytosols, the **sulfotransferase**-mediated metabolic activation of N-OH-ABP was determined as the PAPS-dependent covalent binding of the N-OH substrate to DNA. With cytosols prepared from various tissues, we found that the **sulfotransferase**(s) in human liver, and to a lesser extent colon, can readily metabolize N-OH-ABP. No PAPS-dependent metabolic activation was detected with cytosols prepared from human pancreas or from the carcinogen target tissue, the urinary bladder epithelium. The N-OH-ABP **sulfotransferase** activities of liver and colon cytosols from

different individuals were highly correlated with their thermostable **phenol sulfotransferase** (TS-PST) activity (liver, $r = 0.99$, $P < 0.01$; colon, $r = 0.88$, $P < 0.01$), but not with activities for the thermolabile **phenol sulfotransferase** (TL-PST; liver, $r = 0.29$; colon, $r = 0.53$), or for the dehydroepiandrosterone **sulfotransferase** (DHEA-ST; liver, $r = 0.32$; colon, negligible activity), N-OH-ABP **sulfotransferase** activity was highly sensitive to inhibition by a selective TS-PST inhibitor, 2,6-dichloro-4-nitrophenol ($IC_{50} = 0.7 \mu M$), and by p-nitrophenol, but was unaffected by competitive inhibitors of TL-PST (dopamine) or DHEA-ST (DHEA, DHEA-sulfate). The N-OH-ABP **sulfotransferase** activity also exhibited thermostability properties similar to that of the TS-PST. From these data, we conclude that human liver TS-PST but not TL-PST or DHEA-ST can metabolically activate the proximate human carcinogen N-OH-ABP to a reactive sulfuric acid ester intermediate that binds covalently to DNA. In addition, in view of the putative role of N-OH-ABP as a major transport form of the carcinogen to the urinary bladder and of the absence of **sulfotransferase** activity in this tissue, we hypothesize that **sulfotransferase** activation in the liver may actually decrease the bioavailability of N-OH-ABP toward extrahepatic tissues and thus serve as an important overall detoxification mechanism for the urinary bladder.

L182 ANSWER 21 OF 21 Elsevier BIOBASE COPYRIGHT 2002 Elsevier Science B.V.
 AB Sulfation plays an obligatory role in the activation of N-hydroxy derivatives of carcinogenic arylamine(amide)s and heterocyclic amines. We found that the hepatic sulfotransferase-mediated covalent binding of ³H-labeled 2-hydroxyamino-1-methyl-6-phenylimidazo 4,5-b pyridine (N-OH-PhIP) to calf thymus DNA was 3.3 and 12.9 times higher with human cytosol preparation than with male and female rat cytosol preparations, respectively, in the presence of 3'-phosphoadenosine 5'-phosphosulfate. To assess the activating capacities of individual **phenol**-sulfating **sulfotransferases**, five different forms, human ST1A2 and ST1A3 and rat ST1A1, ST1B1 and ST1C1, were expressed in heterologous cells. All five sulfotransferases mediated the activation of N-OH-PhIP to DNA-bound products. The extents of the binding, however, differed considerably among these forms. Human ST1A2 and ST1A3 mediated the activation of N-OH-PhIP at 5.2- and 6.2-fold higher rates than did rat ST1C1, a main N-hydroxy-2-acetylaminofluorene-activating sulfotransferase, in rat liver. Extents of the binding of N-OH-PhIP in human hepatic cytosols of different individuals were positively correlated with the contents of immunoreactive ST1A2/3. These results suggest a potential role of human liver sulfotransferases in N-OH-PhIP activation. In contrast, the low sulfotransferase-mediated activation of N-OH-PhIP in rat liver is consistent with the lack of PhIP hepatocarcinogenicity in this species.

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

159.50

159.71

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

-0.62

-0.62

STN INTERNATIONAL LOGOFF AT 12:27:02 ON 07 OCT 2002

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	28	sulfotransferase\$1 NEAR5 PHENOL\$8	USPAT; US-PGPUB	2002/10/07 11:08
2	L2	14	sulfotransferase\$1 AND PST	USPAT; US-PGPUB	2002/10/07 11:09
3	L3	35	1 or 2	USPAT; US-PGPUB	2002/10/07 11:09

PGPUB-DOCUMENT-NUMBER: 20020142981

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020142981 A1

TITLE: Gene expression profiles in liver cancer

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Horne, Darci T.	Gaithersburg	MD	US	
Scherf, Uwe	Gaithersburg	MD	US	
Vockley, Joseph	Damascus	MD	US	

US-CL-CURRENT: 514/44;435/6

ABSTRACT:

The present invention identifies the global changes in gene expression associated with liver cancer by examining gene expression in tissue from normal liver, metastatic malignant liver and hepatocellular carcinoma. The present invention also identifies expression profiles which serve as useful diagnostic markers as well as markers that can be used to monitor disease states, disease progression, drug toxicity, drug efficacy and drug metabolism.

PGPUB-DOCUMENT-NUMBER: 20020123612

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020123612 A1

TITLE: Novel human proteins, polynucleotides encoding them and methods of using the same

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gerlach, Valerie L.	Branford	CT	US	
Ellerman, Karen	Branford	CT	US	
MacDougall, John R.	Hamden	CT	US	
Smithson, Glennda	Guilford	CT	US	

US-CL-CURRENT: 530/350;435/320.1 ;435/325 ;435/69.1 ;536/23.5

ABSTRACT:

The invention provides polypeptides, designated herein as POLYX polypeptides, as well as polynucleotides encoding POLYX polypeptides, and antibodies that immunospecifically-bind to POLYX polypeptide or polynucleotide, or derivatives, variants, mutants, or fragments thereof. The invention additionally provides methods in which the POLYX polypeptide, polynucleotide, and antibody are used in the detection, prevention, and treatment of a broad range of pathological states.

PGPUB-DOCUMENT-NUMBER: 20020119462

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020119462 A1

TITLE: Molecular toxicology modeling

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mendrick, Donna L.	Mount Airy	MD	US	
Porter, Mark W.	Germantown	MD	US	
Johnson, Kory R.	Bethesda	MD	US	
Castle, Arthur L.	Washington	DC	US	
Elashoff, Michael R.	Germantown	MD	US	

US-CL-CURRENT: 435/6;702/20

ABSTRACT:

The present invention is based on the elucidation of the global changes in gene expression and the identification of toxicity markers in tissues or cells exposed to a known toxin. The genes may be used as toxicity markers in drug screening and toxicity assays. The invention includes a database of genes characterized by toxin-induced differential expression that is designed for use with microarrays and other solid-phase probes.

PGPUB-DOCUMENT-NUMBER: 20020091087

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020091087 A1

TITLE: Prevention and treatment of degenerative diseases by glutathione and phase II detoxification enzymes

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zhang, Yuesheng	Tucson	AZ	US	
Ho, Tony W.	Malvern	PA	US	
Li, Yun	Tucson	AZ	US	

US-CL-CURRENT: 514/18;514/23 ;514/506 ;514/717 ;514/731 ;514/733

ABSTRACT:

The present invention generally relates to the field of treating degenerative disease by administering a pharmaceutically effective amount of a compound that elevates glutathione or at least one Phase II detoxification enzyme in diseased tissue. The present invention also relates to a pharmaceutical composition useful for the treatment of degenerative diseases, as well as a method of identifying agents that modulate intracellular levels of glutathione or intracellular levels of at least one Phase II enzyme in neuronal cells.

PGPUB-DOCUMENT-NUMBER: 20020085995

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020085995 A1

TITLE: In vivo induction for enhanced function of isolated hepatocytes

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sullivan, Susan J.	Newton	MA	US	
Gregory, Paul G.	Shrewsbury	MA	US	
DiMilla, Paul A.	Dover	MA	US	

US-CL-CURRENT: 424/93.7;435/325

ABSTRACT:

The invention features a liver cell culture comprising hepatocytes that have increased detoxification enzyme activity when isolated from a liver of a donor that had been administered at least one induction agent prior isolation of hepatocyte cells. The induced hepatocytes are used in a bioreactor and cultured to produce hepatocyte cell products or metabolize toxins added to the culture. The bioreactor is, or is an integral part of, a liver assist device used to treat a patient in need of liver assist.

PGPUB-DOCUMENT-NUMBER: 20020073446

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020073446 A1

TITLE: Genetically modified plants having modulated brassinosteroid signaling

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Neff, Michael M.	St. Louis	MO	US	
Chory, Joanne	Del Mar	CA	US	

US-CL-CURRENT: 800/278;536/23.6 ;800/290

ABSTRACT:

Disclosed are plants and methods of making plants that have a dwarf stature in comparison to wildtype plants. By transforming a wildtype plant with a "bas1" gene, or functional homolog thereof, the plant grows to have a dwarf stature.

PGPUB-DOCUMENT-NUMBER: 20020072059

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020072059 A1

TITLE: Method of identifying cells using DNA methylation patterns

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Shiota, Kunio	Chiba		JP	
Tanaka, Satoshi	Saitama		JP	
Ohgane, Jun	Tokyo		JP	
Hattori, Naka	Tokyo		JP	

US-CL-CURRENT: 435/6;702/20

ABSTRACT:

The present invention provides a method of identifying a cell, tissue or nucleus, comprising collecting information on the methylation pattern of DNA isolated from the cell, tissue or nucleus and analyzing the resultant information.

PGPUB-DOCUMENT-NUMBER: 20020039775

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020039775 A1

TITLE: Sulfotransferase sequence variants

PUBLICATION-DATE: April 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Weinshilboum, Richard	Rochester	MN	US	
M.	Elkins Park	PA	US	
Raftogianis, Rebecca B.	Rochester	MN	US	
Wood, Thomas C.	Rochester	MN	US	
Otterness, Diane M.				

US-CL-CURRENT: 435/193;435/6 ;435/69.1 ;536/23.2

ABSTRACT:

Isolated sulfotransferase nucleic acid molecules that include a nucleotide sequence variant and nucleotides flanking the sequence variant are described. Methods for determining a risk estimate for hormone dependent disease and methods for determining sulfonator status also are described.

PGPUB-DOCUMENT-NUMBER: 20020022255

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020022255 A1

TITLE: Transgenic mice containing sulfotransferase gene disruptions

PUBLICATION-DATE: February 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Allen, Keith D.	Cary	NC	US	
Phillips, Russell	Menlo Park	CA	US	

US-CL-CURRENT: 435/193;435/320.1 ;435/325 ;800/18

ABSTRACT:

The present invention relates to transgenic animals, as well as compositions and methods relating to the characterization of gene function. Specifically, the present invention provides transgenic mice comprising disruptions in sulfotransferase genes. Such transgenic mice are useful as models for disease and for identifying agents that modulate gene expression and gene function, and as potential treatments for various disease states and disease conditions.

PGPUB-DOCUMENT-NUMBER: 20020016980

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020016980 A1

TITLE: Transgenic plants incorporating traits of *zostera marina*

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Alberte, Randall S.	Falmouth	ME	US	
Smith, Robert	Falmouth	ME	US	

US-CL-CURRENT: 800/289;536/23.6 ;800/278

ABSTRACT:

The invention provides methods and compositions related to transgenic plants which incorporate genetic traits of the marine eelgrass *Zostera marina*. These traits include pathogen resistance, which may be conferred by stimulating zosteric acid biosynthesis, and root anoxia resistance, which may be conferred by introducing one or more anoxia-induced or anoxia-resistance genes.

PGPUB-DOCUMENT-NUMBER: 20020009730

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020009730 A1

TITLE: Human stress array

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chenchik, Alex	Palo Alto	CA	US	
Lukashev, Matvey E.	Newton	MA	US	

US-CL-CURRENT: 435/6;536/24.3

ABSTRACT:

Human stress arrays and methods for their use are provided. The subject arrays include a plurality of polynucleotide spots, each of which is made up of a polynucleotide probe composition of unique polynucleotides corresponding to a human stress gene. The subject arrays find use in hybridization assays, particularly in assays for the identification of differential gene expression of human stress genes.

PGPUB-DOCUMENT-NUMBER: 20010051370

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010051370 A1

TITLE: Glycosyl sulfotransferase-3

PUBLICATION-DATE: December 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bistrup, Annette	San Francisco	CA	US	
Rosen, Steven D.	San Francisco	CA	US	
Hemmerich, Stefan	Berkeley	CA	US	

US-CL-CURRENT: 435/193;435/320.1 ;536/23.2

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith.

PGPUB-DOCUMENT-NUMBER: 20010034023

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010034023 A1

TITLE: Gene sequence variations with utility in determining the treatment of disease, in genes relating to drug processing

PUBLICATION-DATE: October 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stanton, Vincent P. JR.	Belmont	MA	US	
Zillmann, Martin	Shrewsbury	MA	US	

US-CL-CURRENT: 435/6;702/20

ABSTRACT:

Methods for identifying and utilizing variances in genes relating to efficacy and safety of medical therapy and other aspects of medical therapy are described, including methods for selecting an effective treatment.

US-PAT-NO: 6455289

DOCUMENT-IDENTIFIER: US 6455289 B1

TITLE: Polypeptide of N-acetylglucosamine-6-O-sulfotransferase and DNA encoding the same

DATE-ISSUED: September 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Uchimura; Kenji	Nagoya	N/A	N/A	JP
Muramatsu; Hideki	Shizuoka-ken	N/A	N/A	JP
Kadomatsu; Kenji	Nagoya	N/A	N/A	JP
Kannagi; Reiji	Nagoya	N/A	N/A	JP
Habuchi; Osami	Nagoya	N/A	N/A	JP
Muramatsu; Takashi	Nagoya	N/A	N/A	JP

US-CL-CURRENT: 435/193; 435/183 ; 435/194 ; 530/350

ABSTRACT:

Apolypeptide of N-acetylglucosamine-6-O-sulfotransferase and a DNA encoding the peptide are provided. The polypeptide is (a) or (b) below: (a) a polypeptide consisting of an amino acid sequence represented by SEQ ID NO: 2; or (b) a polypeptide which comprises an amino acid sequence including substitution, deletion, insertion or transposition of one or few amino acids in the amino acid sequence of (a) and which has an enzymatic activity to transfer a sulfate group from a sulfate group donor to a hydroxyl group at 6 position of an N-acetylglucosamine residue located at a non-reducing end of an oligosaccharide represented by the formula I:

GlcNAc.beta.1-3Gal.beta.1-4GlcNAc (I)

wherein GlcNAc represents an N-acetylglucosamine residue, Gal represents a galactose residue, .beta.1-3 represents a .beta.1-3 glycosidic linkage, and .beta.1-4 represents a .beta.1-4 glycosidic linkage.

4 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

US-PAT-NO: 6448003

DOCUMENT-IDENTIFIER: US 6448003 B1

TITLE: Genotyping the human phenol sulfotransferase 2 gene STP2

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Guida; Marco	San Diego	CA	N/A	N/A
Kurth; Janice	San Diego	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/91.2 ; 536/23.2 ; 536/23.5 ; 536/24.31 ; 536/24.33

ABSTRACT:

Genetic polymorphisms are identified in the human STP2 gene that alter STP2-dependent drug metabolism. Nucleic acids comprising the polymorphic sequences are used to screen patients for altered metabolism for STP2 substrates, potential drug-drug interactions, and adverse/side effects, as well as diseases that result from environmental or occupational exposure to toxins. The nucleic acids are used to establish animal, cell and in vitro models for drug metabolism.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 5

US-PAT-NO: 6436684

DOCUMENT-IDENTIFIER: US 6436684 B1

TITLE: Isolated human drug-metabolizing proteins, nucleic acid molecules encoding human drug-metabolizing proteins, and uses thereof

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Woodage; Trevor	Washington	DC	N/A	N/A
Wei; Ming-Hui	Germantown	MD	N/A	N/A
Kodira; Chinnappa	Germantown	MD	N/A	N/A
Di Francesco; Valentina	Rockville	MD	N/A	N/A
Beasley; Ellen M.	Darnestown	MD	N/A	N/A

US-CL-CURRENT: 435/193; 435/252.3 ; 435/320.1 ; 435/6 ; 536/23.2

ABSTRACT:

The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the proteins of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the proteins of the present invention, and methods of identifying modulators of the proteins of the present invention.

11 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

US-PAT-NO: 6394812

DOCUMENT-IDENTIFIER: US 6394812 B1

TITLE: Vivo induction for enhanced function of isolated hepatocytes

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sullivan; Susan J.	Newton	MA	N/A	N/A
Gregory; Paul G.	Shrewsbury	MA	N/A	N/A
DiMilla; Paul A.	Dover	MA	N/A	N/A

US-CL-CURRENT: 434/375; 424/93.7

ABSTRACT:

The invention features a liver cell culture comprising hepatocytes that have increased detoxification enzyme activity when isolated from a liver of a donor that had been administered at least one induction agent prior isolation of hepatocyte cells. The induced hepatocytes are used in a bioreactor and cultured to produce hepatocyte cell products or metabolize toxins added to the culture. The bioreactor is, or is an integral part of, a liver assist device used to treat a patient in need of liver assist.

20 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

US-PAT-NO: 6365365

DOCUMENT-IDENTIFIER: US 6365365 B1

TITLE: Method of determining whether an agent modulates glycosyl sulfotransferase-3

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bistrup; Annette	San Francisco	CA	N/A	N/A
Rosen; Steven D.	San Francisco	CA	N/A	N/A
Tangemann; Kirsten	Menlo Park	CA	N/A	N/A
Hemmerich; Stefan	Berkeley	CA	N/A	N/A

US-CL-CURRENT: 435/15; 435/14 ; 435/194 ; 530/350 ; 536/23.1

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith, particularly by administering an inhibitor of at least one of GST-3 or KSGal6ST, or homologues thereof.

31 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

US-PAT-NO: 6335170

DOCUMENT-IDENTIFIER: US 6335170 B1

TITLE: Gene expression in bladder tumors

DATE-ISSUED: January 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Orntoft; Torben F.	DK 8230 Aabyhoj	N/A	N/A	DK

US-CL-CURRENT: 435/6; 435/91.1 ; 435/91.2 ; 536/23.1 ; 536/24.3 ; 536/24.31 ; 536/24.33

ABSTRACT:

Methods for analyzing tumor cells, particularly bladder tumor cells employ gene expression analysis of samples. Gene expression patterns are formed and compared to reference patterns. Alternatively gene expression patterns are manipulated to exclude genes which are expressed in contaminating cell populations. Another alternative employs subtraction of the expression of genes which are expressed in contaminating cell types. These methods provide improved accuracy as well as alternative basis for analysis from diagnostic and prognostic tools currently available.

21 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

US-PAT-NO: 6313268

DOCUMENT-IDENTIFIER: US 6313268 B1

TITLE: Secretases related to Alzheimer's dementia

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hook; Vivian Y. H.	La Jolla	CA	92037	N/A

US-CL-CURRENT: 530/350; 424/563 ; 424/570 ; 424/94.1 ; 424/94.2 ; 424/94.6
; 424/94.63 ; 424/94.66 ; 435/183 ; 435/212 ; 435/226 ; 530/412 ; 530/422
; 530/427

ABSTRACT:

This invention is directed to a novel .beta.-secretase that produces the A.beta. peptide found in Alzheimer's Disease. One .beta.-secretase is a protein having a molecular weight of about 61, 81 or 88 kDa that cleaves an amyloid precursor protein (APP) substrate. Another is a protease complex having a molecular between about 180 and 200 kDa, which, in one embodiment, contains the 61, 81, and 88 kDa proteins and, in another embodiment, contains proteins having a molecular weight of about 66, 60, 33 and 29 kDa. Another .beta.-secretase has a molecular weight between about 50 and 90 kDa. The invention is also directed to methods of selecting agents that inhibit A.beta. peptide production and treating Alzheimer's disease in patients.

11 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

US-PAT-NO: 6265561

DOCUMENT-IDENTIFIER: US 6265561 B1

TITLE: Sulfotransferase sequence variants

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weinshilbourn; Richard	Rochester	MN	N/A	N/A
M.	Elkins Park	PA	N/A	N/A
Raftogianis; Rebecca B.	Rochester	MN	N/A	N/A
Wood; Thomas C.	Rochester	MN	N/A	N/A
Otterness; Diane M.				

US-CL-CURRENT: 536/23.2; 435/193 ; 536/23.1

ABSTRACT:

Isolated sulfotransferase nucleic acid molecules that include a nucleotide sequence variant and nucleotides flanking the sequence variant are described. Methods for determining a risk estimate for hormone dependent disease and methods for determining sulfonator status also are described.

3 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

US-PAT-NO: 6265192

DOCUMENT-IDENTIFIER: US 6265192 B1

TITLE: Glycosyl sulfotransferase-3

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bistrup; Annette	San Francisco	CA	N/A	N/A
Rosen; Steven D.	San Francisco	CA	N/A	N/A
Hemmerich; Stefan	Berkeley	CA	N/A	N/A

US-CL-CURRENT: 435/193; 435/183 ; 435/252.3 ; 435/320.1 ; 435/69.1 ; 536/23.2

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith.

7 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

US-PAT-NO: 6245884

DOCUMENT-IDENTIFIER: US 6245884 B1

TITLE: Secretases related to alzheimer's dementia

DATE-ISSUED: June 12, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hook; Vivian Y. H.	La Jolla	CA	92037	N/A

US-CL-CURRENT: 530/300; 424/9.2 ; 435/23 ; 435/29 ; 435/326 ; 435/331 ; 435/332 ; 435/69.2 ; 435/7.1 ; 530/331 ; 530/350 ; 562/545 ; 562/577

ABSTRACT:

This invention provides a method of determining the proteolytic activity of the in vivo secretases, particularly the .beta.-secretase and .gamma.-secretase that produce the A.beta. peptides found in the plaques of Alzheimer Dementia (AD) patients. The invention also provides methods of isolating such secretases and methods of selecting agents that affect the activity of such secretases for developing drugs to treat or prevent dementia.

8 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

US-PAT-NO: 6207432

DOCUMENT-IDENTIFIER: US 6207432 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

US-CL-CURRENT: 435/193; 536/23.2 ; 536/23.5

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

4 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

US-PAT-NO: 6207414

DOCUMENT-IDENTIFIER: US 6207414 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/193 ; 536/23.2

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

2 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

US-PAT-NO: 6204016

DOCUMENT-IDENTIFIER: US 6204016 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/193 ; 435/252.33 ; 435/320.1 ; 435/325 ; 536/23.1 ; 536/23.2 ; 536/23.5

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

27 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

US-PAT-NO: 6160006

DOCUMENT-IDENTIFIER: US 6160006 A

TITLE: 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor in grapefruit

DATE-ISSUED: December 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Edwards; David J.	LaSalle	N/A	N/A	CA
Woster; Patrick M.	Canton	MI	N/A	N/A

US-CL-CURRENT: 514/455; 549/282

ABSTRACT:

The present invention provides a composition and methods for inhibiting cytochrome P450 enzyme activity and in particular, inhibiting the activity of the cytochrome P450 3A sub-family of enzymes, specifically, CYP3A4. The present invention provides 6',7'-dihydroxybergamottin, a furanocoumarin, as the compound primarily responsible for the inhibitory effects of grapefruit juice on cytochrome P450 enzyme activity. The present invention also provides a novel synthesis scheme for 6',7'-dihydroxybergamottin.

25 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

US-PAT-NO: 6071732

DOCUMENT-IDENTIFIER: US 6071732 A

TITLE: Tyrosylprotein sulfotransferases, nucleic acids encoding tyrosylprotein sulfotransferases, and methods of use thereof

DATE-ISSUED: June 6, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

US-CL-CURRENT: 435/193

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system.

3 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

US-PAT-NO: 6060295

DOCUMENT-IDENTIFIER: US 6060295 A

TITLE: Nucleic acids and expression systems encoding tyrosylprotein sulfo transferases

DATE-ISSUED: May 9, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

US-CL-CURRENT: 435/193; 435/320.1 ; 435/325 ; 536/23.2

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and C. elegans. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system.

29 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

US-PAT-NO: 6037159

DOCUMENT-IDENTIFIER: US 6037159 A

TITLE: Polypeptide of N-acetylglucosamine-6-O-sulfotransferase and DNA encoding the same

DATE-ISSUED: March 14, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Uchimura; Kenji	Nagoya	N/A	N/A	JP
Muramatsu; Hideki	Shizuoka-ken	N/A	N/A	JP
Kadomatsu; Kenji	Nagoya	N/A	N/A	JP
Kannagi; Reiji	Nagoya	N/A	N/A	JP
Habuchi; Osami	Nagoya	N/A	N/A	JP
Muramatsu; Takashi	Nagoya	N/A	N/A	JP

US-CL-CURRENT: 435/193; 435/183 ; 435/252.1 ; 435/69.1 ; 435/72

ABSTRACT:

Apolypeptide of N-acetylglucosamine-6-O-sulfotransferase and a DNA encoding the peptide are provided. The polypeptide is (a) or (b) below:

(a) a polypeptide consisting of an amino acid sequence represented by SEQ ID NO: 2; or

(b) a polypeptide which comprises an amino acid sequence including substitution, deletion, insertion or transposition of one or few amino acids in the amino acid sequence of (a) and which has an enzymatic activity to transfer a sulfate group from a sulfate group donor to a hydroxyl group at 6 position of an N-acetylglucosamine residue located at a non-reducing end of an oligosaccharide represented by the formula I:

GlcNAc.beta.1-3Gal.beta.1-4GlcNAc (I)

wherein GlcNAc represents an N-acetylglucosamine residue, Gal represents a galactose residue, .beta.1-3 represents a .beta.1-3 glycosidic linkage, and .beta.1-4 represents a .beta.1-4 glycosidic linkage.

13 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

US-PAT-NO: 5744355

DOCUMENT-IDENTIFIER: US 5744355 A

TITLE: cDNA cloning and expression of human liver estrogen sulfotransferase

DATE-ISSUED: April 28, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weinshilbourn; Richard	Rochester	MN	N/A	N/A
M.	Rochester	MN	N/A	N/A
Aksoy; Ibrahim A.	Rochester	MN	N/A	N/A
Wood; Thomas C.				

US-CL-CURRENT: 435/325; 435/193 ; 435/252.3 ; 435/320.1 ; 536/23.1 ; 536/23.2

ABSTRACT:

The present invention provides an isolated and purified human DNA molecule that encodes human estrogen sulfotransferase.

8 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

US-PAT-NO: 5714594

DOCUMENT-IDENTIFIER: US 5714594 A

TITLE: cDNA cloning and expression of human liver estrogen sulfotransferase

DATE-ISSUED: February 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weinshilbourn; Richard	Rochester	MN	N/A	N/A
M.	Rochester	MN	N/A	N/A
Aksoy; Ibrahim A.	Rochester	MN	N/A	N/A
Wood; Thomas C.				

US-CL-CURRENT: 536/23.2; 435/193 ; 435/252.3 ; 435/320.1 ; 536/23.1

ABSTRACT:

The present invention provides an isolated and purified human DNA molecule consisting essentially of a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof.

9 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

US-PAT-NO: 5672584

DOCUMENT-IDENTIFIER: US 5672584 A

TITLE: Cyclic prodrugs of peptides and peptide nucleic acids having improved metabolic stability and cell membrane permeability

DATE-ISSUED: September 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchardt; Ronald T.	Lawrence	KS	N/A	N/A
Siahaan; Teruna	Lawrence	KS	N/A	N/A
Gangwar; Sanjeev	Lawrence	KS	N/A	N/A
Stella; Valentino J.	Lawrence	KS	N/A	N/A
Wang; Binghe	Norman	OK	N/A	N/A

US-CL-CURRENT: 514/11; 530/317

ABSTRACT:

Provided are cyclic prodrugs of biologically active peptides and peptide nucleic acids exhibiting improved cell membrane permeability and enzymatic stability, containing 3-(2'-hydroxy-4',6'-dimethyl phenyl)-3,3-dimethyl propionic acid and its derivatives and acyloxyalkoxy linkers. Also provided are pharmaceutical compositions containing effective amounts of these cyclic prodrugs in combination with pharmaceutically acceptable carriers, excipients, or diluents.

5 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

US-PAT-NO: 5580757

DOCUMENT-IDENTIFIER: US 5580757 A

TITLE: Cloning and expression of biologically active .alpha.-galactosidase A as a fusion protein

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Desnick; Robert J.	New York	NY	N/A	N/A
Bishop; David F.	New York	NY	N/A	N/A
Ioannou; Yiannis A.	New York	NY	N/A	N/A

US-CL-CURRENT: 435/69.7; 435/208 ; 435/320.1

ABSTRACT:

The present invention involves the production of large quantities of human .alpha.-Gal A by cloning and expressing the .alpha.-Gal A coding sequence in eukaryotic host cell expression systems. The eukaryotic expression systems, and in particular the mammalian host cell expression system described herein provide for the appropriate cotranslational and posttranslational modifications required for proper processing, e.g., glycosylation, phosphorylation, etc. and sorting of the expression product so that an active enzyme is produced. In addition, the expression of fusion proteins which simplify purification is described.

Using the methods described herein, the recombinant .alpha.-Gal A is secreted by the engineered host cells so that it is recovered from the culture medium in good yield. The .alpha.-Gal A produced in accordance with the invention may be used, but is not limited to, in the treatment in Fabry Disease; for the hydrolysis of .alpha.-galactosyl residues in glycoconjugates; and/or for the conversion of the blood group B antigen on erythrocytes to the blood group O antigen.

15 Claims, 51 Drawing figures

Exemplary Claim Number: 5

Number of Drawing Sheets: 38

US-PAT-NO: 5401650

DOCUMENT-IDENTIFIER: US 5401650 A

TITLE: Cloning and expression of biologically active .alpha.-galactosidase A

DATE-ISSUED: March 28, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Desnick; Robert J.	New York	NY	N/A	N/A
Bishop; David F.	New York	NY	N/A	N/A
Ioannou; Yiannis A.	New York	NY	N/A	N/A

US-CL-CURRENT: 435/208; 435/193 ; 536/23.2 ; 536/23.4

ABSTRACT:

The present invention involves the production of large quantities of human .alpha.-Gal A by cloning and expressing the .alpha.-Gal A coding sequence in eukaryotic host cell expression systems. The eukaryotic expression systems, and in particular the mammalian host cell expression system described herein provide for the appropriate cotranslational and posttranslational modifications required for proper processing, e.g., glycosylation, phosphorylation, etc. and sorting of the expression product so that an active enzyme is produced. In addition, the expression of fusion proteins which simplify purification is described.

Using the methods described herein, the recombinant .alpha.-Gal A is secreted by the engineered host cells so that it is recovered from the culture medium in good yield. The .alpha.-Gal A produced in accordance with the invention may be used, but is not limited to, in the treatment in Fabry Disease; for the hydrolysis of .alpha.-galactosyl residues in glycoconjugates; and/or for the conversion of the blood group B antigen on erythrocytes to the blood group O antigen.

9 Claims, 51 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 38

PGPUB-DOCUMENT-NUMBER: 20010051370

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010051370 A1

TITLE: Glycosyl sulfotransferase-3

PUBLICATION-DATE: December 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bistrup, Annette	San Francisco	CA	US	
Rosen, Steven D.	San Francisco	CA	US	
Hemmerich, Stefan	Berkeley	CA	US	

APPL-NO: 09/ 816825

DATE FILED: March 22, 2001

RELATED-US-APPL-DATA:

child 09816825 A1 20010322 parent continuation-of 09045284 19980320 US GRANTED
parent-patent 6265192 US

US-CL-CURRENT: 435/193,435/320.1 ,536/23.2

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0002] Sulfotransferases are enzymes that catalyze the transfer of a sulfate from a donor compound to an acceptor compound, usually placing the sulfate moiety at a specific location on the acceptor compound. There are a variety of different sulfotransferases which vary in activity, i.e. with respect to the donor and/or acceptor compounds with which they work. Known sulfotransferases include those acting on carbohydrate: heparin/heparan sulfate N-sulfotransferase (NST); chondroitin 6/keratan 6 sulfate sulfotransferase

(C6ST/KSST); galactosylceramide 3'-sulfotransferase; heparan sulfate 2-sulfotransferase (Iduronic acid); HNK-1 sulfotransferase (3-glucuronic acid); heparan sulfate D-glucosamino 3-O-sulfotransferase (3-OST); etc., as well as those acting on **phenols, steroids and xenobiotics: aryl sulfotransferase I & II**, hydroxy-steroid sulfotransferases I, II & III, dehydroepiandrosterone (DHEA); etc. Sulfotransferases play a central role in a variety of different biochemical mechanisms, as the presence of a sulfate moiety on a particular ligand is often required for a particular activity, e.g. binding.

US-PAT-NO: 6448003

DOCUMENT-IDENTIFIER: US 6448003 B1

TITLE: Genotyping the human phenol sulfotransferase 2 gene STP2

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Guida; Marco	San Diego	CA	N/A	N/A
Kurth; Janice	San Diego	CA	N/A	N/A

APPL-NO: 09/ 328174

DATE FILED: June 8, 1999

PARENT-CASE:

This application claims the benefit of U.S. Provisional application 60/088,710, filed Jun. 10, 1998.

US-CL-CURRENT: 435/6; 435/91.2 ; 536/23.2 ; 536/23.5 ; 536/24.31 ; 536/24.33

ABSTRACT:

Genetic polymorphisms are identified in the human STP2 gene that alter STP2-dependent drug metabolism. Nucleic acids comprising the polymorphic sequences are used to screen patients for altered metabolism for STP2 substrates, potential drug-drug interactions, and adverse/side effects, as well as diseases that result from environmental or occupational exposure to toxins. The nucleic acids are used to establish animal, cell and in vitro models for drug metabolism.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 5

----- KWIC -----

Brief Summary Text - BSTX:

Sulfonation is an important pathway in the biotransformation of many drugs, xenobiotics, neurotransmitters, and steroid hormones. Many of the sulfonation reactions for pharmacologic agents are performed by a group of enzymes known as phenol transferases. The phenol sulfotransferase gene family consists to three members located on chromosome 16. A single gene (STM) encodes the thermolabile monoamine-metabolizing form. Two thermostable phenol-metabolizing enzymes are

encoded by STP1 and STP2. Substrates for STP1 and STP2 include minoxidil, acetaminophen, and para-nitrophenol. Alterations in phenol sulfotransferase activity have been correlated with individual variation in sulfonation of acetaminophen (Reiter and Weinshilboum (1982) Clin. Pharm.) and predisposition to diet-induced migraine headaches.

Brief Summary Text - BSTX:

Since rates of metabolism of drugs, toxins, etc. can depend on the amounts and kinds of phenol sulfotransferase in tissues, variation in biological response may be determined by the profile of expression of phenol sulfotransferases in each person. Analysis of genetic polymorphisms that lead to altered expression and/or enzyme activity are therefore of interest.

US-PAT-NO: 6365365

DOCUMENT-IDENTIFIER: US 6365365 B1

TITLE: Method of determining whether an agent modulates glycosyl sulfotransferase-3

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bistrup; Annette	San Francisco	CA	N/A	N/A
Rosen; Steven D.	San Francisco	CA	N/A	N/A
Tangemann; Kirsten	Menlo Park	CA	N/A	N/A
Hemmerich; Stefan	Berkeley	CA	N/A	N/A

APPL-NO: 09/ 190911

DATE FILED: November 12, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of application Ser. No. 09/045,284 filed on Mar. 20, 1998, the disclosure of which is herein incorporated by reference.

US-CL-CURRENT: 435/15; 435/14 ; 435/194 ; 530/350 ; 536/23.1

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith, particularly by administering an inhibitor of at least one of GST-3 or KSGal6ST, or homologues thereof.

31 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

----- KWIC -----

Brief Summary Text - BSTX:

Sulfotransferases are enzymes that catalyze the transfer of a sulfate from a donor compound to an acceptor compound, usually placing the sulfate moiety at a specific location on the acceptor compound. There are a variety of different sulfotransferases which vary in activity, i.e. with respect to the donor and/or acceptor compounds with which they work. Known sulfotransferases include those acting on carbohydrate: heparin/heparan sulfate N-sulfotransferase (NST); chondroitin 6/keratan 6 sulfate sulfotransferase (C6ST/KSST); galactosylceramide 3'-sulfotransferase; heparan sulfate 2-sulfotransferase (Iduronic acid); HNK-1 sulfotransferase (3-glucuronic acid); heparan sulfate D-glucosamino 3-O-sulfotransferase (3-OST); etc., as well as those acting on **phenols, steroids and xenobiotics: aryl sulfotransferase I & II**, hydroxy-steroid sulfotransferases I, II & III, dehydroepiandrosterone (DHEA); etc. Sulfotransferases play a central role in a variety of different biochemical mechanisms, as the presence of a sulfate moiety on a particular ligand is often required for a particular activity, e.g. binding.

US-PAT-NO: 6265192

DOCUMENT-IDENTIFIER: US 6265192 B1

TITLE: Glycosyl sulfotransferase-3

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bistrup; Annette	San Francisco	CA	N/A	N/A
Rosen; Steven D.	San Francisco	CA	N/A	N/A
Hemmerich; Stefan	Berkeley	CA	N/A	N/A

APPL-NO: 09/ 045284

DATE FILED: March 20, 1998

US-CL-CURRENT: 435/193; 435/183 ; 435/252.3 ; 435/320.1 ; 435/69.1 ; 536/23.2

ABSTRACT:

A novel human glycosylsulfotransferase expressed in high endothelial cells (GST-3) and polypeptides related thereto, as well as nucleic acid compositions encoding the same, are provided. The subject polypeptides and nucleic acid compositions find use in a variety of applications, including research, diagnostic, and therapeutic agent screening applications. Also provided are methods of inhibiting selectin mediated binding events and methods of treating disease conditions associated therewith.

7 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Brief Summary Text - BSTX:

Sulfotransferases are enzymes that catalyze the transfer of a sulfate from a donor compound to an acceptor compound, usually placing the sulfate moiety at a specific location on the acceptor compound. There are a variety of different sulfotransferases which vary in activity, i.e. with respect to the donor and/or acceptor compounds with which they work. Known sulfotransferases include those acting on carbohydrate: heparin/heparan sulfate N-sulfotransferase (NST); chondroitin 6/keratan 6 sulfate sulfotransferase (C6ST/KSST); galactosylceramide 3'-sulfotransferase; heparan sulfate 2-sulfotransferase

(Iduronic acid); 14NK-1 sulfotransferase (3-glucuronic acid); heparan sulfate D-glucosamino 3-O-sulfotransferase (3-OST);etc., as well as those acting on **phenols, steroids and xenobiotics: aryl sulfotransferase** I & II, hydroxy-steroid sulfotransferases I, II & III, dehydroepiandrosterone (DHEA); etc. Sulfotransferases play a central role in a variety of different biochemical mechanisms, as the presence of a sulfate moiety on a particular ligand is often required for a particular activity, e.g. binding.

US-PAT-NO: 6207432

DOCUMENT-IDENTIFIER: US 6207432 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

APPL-NO: 09/ 374492

DATE FILED: August 13, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a continuation-in-part of U.S. Ser. No. 09/150,141, filed Sep. 9, 1998, now U.S. Pat. No. 6,071,732 which claims the benefit of U.S. Provisional Application Ser. No. 60/072,994, filed Jan. 29, 1998. The present application is also a continuation-in-part of International Application No. PCT/US99/16750 filed Jul. 23, 1999.

US-CL-CURRENT: 435/193; 536/23.2 ; 536/23.5

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

4 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

TPST-1 and other TPSTs exhibit homology to a large family of cytosolic sulfotransferases, including phenol- and hydroxysteroid-sulfotransferases. The known members of this family contain two regions which are highly conserved throughout phlogeny, called region I and region IV (31). These regions are involved in binding of the sulfate donor PAPS (32-35). Alignment of mouse TPST-1 and mouse estrogen sulfotransferase reveals a 20% identity and 52% similarity with 19 alignment gaps, 12 of which are 3 residues in length (FIG. 6). Notably, TPST-1 has a 35 residue amino terminal extension that includes the putative non-cleavable signal peptide/membrane anchor. In the estrogen sulfotransferase crystal structure, the residues which contact the 5' phosphate of PAPS (PKSGTTW (SEQ ID NO:44)) form a loop between .beta.-sheet 3 and .alpha.-helix 3, which corresponds to region I (35). This region is highly conserved in TPST-1, TPST-2, TPST-A, and TPST-B, and corresponds to residues 78-84 (PRSGTTL (SEQ ID NO:45)) in TPST-1, residues 77-83 in TPST-2, residues 78-84 in TPST-A, and residues 94-100 in TPST-B (see FIG. 13). The residues involved in binding the 3' phosphate of PAP are located in two discontinuous regions of estrogen sulfotransferase. The first region includes two residues, Arg.sup.130 and Ser.sup.138, located just before and within .alpha.-helix 6. The second is comprised of residues 257-259 (Arg-Lys-Gly). The corresponding residues in TPST-1 are Arg.sup.184, Ser.sup.192, Ala.sup.322, Lys.sup.323, and Leu.sup.324. Thus, although the degree of identity is limited, most of the residues involved in PAPS binding in the estrogen sulfotransferase (SEQ ID NO:13) structure are predicted to be conserved in TPST (FIG. 6). TPST exhibits a similar degree of homology to Golgi sulfotransferases, including heparan sulfate 2-sulfotransferase (36), chondroitin 6-sulfotransferase (37), and the C-terminal domain of heparan sulfate N-deacetylase/N-sulfotransferase (38).

US-PAT-NO: 6207414

DOCUMENT-IDENTIFIER: US 6207414 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

APPL-NO: 09/ 374824

DATE FILED: August 13, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a continuation-in-part of U.S. Ser. No. 09/150,133, filed Sep. 9, 1998, now U.S. Pat. No. 6,060,295 which claims the benefit of U.S. Provisional Application Ser. No. 60/072,994, filed Jan. 29, 1998. The present application is also a continuation-in-part of International Application No. PCT/US99/16750 filed Jul. 23, 1999.

US-CL-CURRENT: 435/69.1; 435/193 ; 536/23.2

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and C. elegans. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

2 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

TPST-1 and other TPSTs exhibit homology to a large family of cytosolic sulfotransferases, including phenol- and hydroxysteroid-sulfotransferases. The known members of this family contain two regions which are highly conserved throughout phlogeny, called region I and region IV (31). These regions are involved in binding of the sulfate donor PAPS (32-35). Alignment of mouse TPST-1 and mouse estrogen sulfotransferase reveals a 20% identity and 52% similarity with 19 alignment gaps, 12 of which are 3 residues in length (FIG. 6). Notably, TPST-1 has a 35 residue amino terminal extension that includes the putative non-cleavable signal peptide/membrane anchor. In the estrogen sulfotransferase crystal structure, the residues which contact the 5' phosphate of PAPS (PKSGTTW (SEQ ID NO:44)) form a loop between .beta.-sheet 3 and .alpha.-helix 3, which corresponds to region I (35). This region is highly conserved in TPST-1, TPST-2, TPST-A, and TPST-B, and corresponds to residues 78-84 (PRSGTTL (SEQ ID NO:45)) in TPST-1, residues 77-83 in TPST-2, residues 78-84 in TPST-A, and residues 94-100 in TPST-B (see FIG. 13). The residues involved in binding the 3' phosphate of PAP are located in two discontinuous regions of estrogen sulfotransferase. The first region includes two residues, Arg.sup.130 and Ser.sup.138, located just before and within .alpha.-helix 6. The second is comprised of residues 257-259 (Arg-Lys-Gly). The corresponding residues in TPST-1 are Arg.sup.184, Ser.sup.192, Ala.sup.322, Lys.sup.323, and Leu.sup.324. Thus, although the degree of identity is limited, most of the residues involved in PAPS binding in the estrogen sulfotransferase (SEQ ID NO:13) structure are predicted to be conserved in TPST (FIG. 6). TPST exhibits a similar degree of homology to Golgi sulfotransferases, including heparan sulfate 2-sulfotransferase (36), chondroitin 6-sulfotransferase (37), and the C-terminal domain of heparan sulfate N-deacetylase/N-sulfotransferase (38).

US-PAT-NO: 6204016

DOCUMENT-IDENTIFIER: US 6204016 B1

TITLE: Tyrosylprotein sulfotransferases and methods of use thereof

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

APPL-NO: 09/ 374493

DATE FILED: August 13, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a continuation-in-part of U.S. Ser. No. 09/150,133, filed Sep. 9, 1998, now U.S. Pat. No. 6,060,295 which claims the benefit of U.S. Provisional Application Serial No. 60/072,994, filed Jan. 29, 1998. The present application is also a continuation-in-part of International Application No. PCT/US99/16750 filed Jul. 23, 1999.

US-CL-CURRENT: 435/69.1; 435/193 ; 435/252.33 ; 435/320.1 ; 435/325 ; 536/23.1 ; 536/23.2 ; 536/23.5

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and C. elegans. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system. The enzymes can be used to sulfate peptides or proteins requiring sulfation.

27 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

TPST-1 and other TPSTs exhibit homology to a large family of cytosolic **sulfotransferases, including phenol- and hydroxysteroid-sulfotransferases**. The known members of this family contain two regions which are highly conserved throughout phlogeny, called region I and region IV (31). These regions are involved in binding of the sulfate donor PAPS (32-35). Alignment of mouse TPST-1 and mouse estrogen sulfotransferase reveals a 20% identity and 52% similarity with 19 alignment gaps, 12 of which are 3 residues in length (FIG. 6). Notably, TPST-1 has a 35 residue amino terminal extension that includes the putative non-cleavable signal peptide/membrane anchor. In the estrogen sulfotransferase crystal structure, the residues which contact the 5' phosphate of PAPS (PKSGTTW (SEQ ID NO:44)) form a loop between .beta.-sheet 3 and .alpha.-helix 3, which corresponds to region I (35). This region is highly conserved in TPST-1, TPST-2, TPST-A, and TPST-B, and corresponds to residues 78-84 (PRSGTTL (SEQ ID NO:45)) in TPST-1, residues 77-83 in TPST-2, residues 78-84 in TPST-A, and residues 94-100 in TPST-B (see FIG. 13). The residues involved in binding the 3' phosphate of PAP are located in two discontinuous regions of estrogen sulfotransferase. The first region includes two residues, Arg.sup.130 and Ser.sup.138, located just before and within .alpha.-helix 6. The second is comprised of residues 257-259 (Arg-Lys-Gly). The corresponding residues in TPST-1 are Arg.sup.184, Ser.sup.192, Ala.sup.322, Lys.sup.323, and Leu.sup.324. Thus, although the degree of identity is limited, most of the residues involved in PAPS binding in the estrogen sulfotransferase (SEQ ID NO:13) structure are predicted to be conserved in TPST (FIG. 6). TPST exhibits a similar degree of homology to Golgi sulfotransferases, including heparan sulfate 2-sulfotransferase (36), chondroitin 6-sulfotransferase (37), and the C-terminal domain of heparan sulfate N-deacetylase/N-sulfotransferase (38).

US-PAT-NO: 6160006

DOCUMENT-IDENTIFIER: US 6160006 A

TITLE: 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor in grapefruit

DATE-ISSUED: December 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Edwards; David J.	LaSalle	N/A	N/A	CA
Woster; Patrick M.	Canton	MI	N/A	N/A

APPL-NO: 08/ 951330

DATE FILED: October 16, 1997

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation of provisional application Ser. Nos. 60/028,961, filed Oct. 18, 1996 and 60/054,332, filed Jun. 24, 1997, herein expressly incorporated by reference.

US-CL-CURRENT: 514/455; 549/282

ABSTRACT:

The present invention provides a composition and methods for inhibiting cytochrome P450 enzyme activity and in particular, inhibiting the activity of the cytochrome P450 3A sub-family of enzymes, specifically, CYP3A4. The present invention provides 6',7'-dihydroxybergamottin, a furanocoumarin, as the compound primarily responsible for the inhibitory effects of grapefruit juice on cytochrome P450 enzyme activity. The present invention also provides a novel synthesis scheme for 6',7'-dihydroxybergamottin.

25 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Detailed Description Text - DETX:

Using isolated pure compound, in vitro studies are conducted with human liver microsomes to determine the specificity, potency and mechanism of inhibitory effects on substrates for the primary cytochrome P450 enzymes involved in

pro-carcinogen activation (including, but not limited to, CYP1A1, CYP1A2, CYP2A6, CYP2D6, CYP2E1 and CYP3A4). Since conjugation may also result in bioreactive metabolites, **phenol sulfotransferase**, UDP-glucuronosyltransferase and glutathione S-transferase activity is measured under control conditions and following incubation with 6',7'-dihydroxybergamottin.

Detailed Description Text - DETX:

Microsomal or cytosolic fractions are prepared from male Sprague-Dawley rats according to established procedures. Raftogianis, R. B. et al., J. Pharmacol. Exp. Ther. 276:602-608 (1996). Cytosolic glutathione S-transferase activity is assessed under control conditions and with varying concentrations of 6',7'-dihydroxybergamottin using 1-chloro-2,4-dinitrobenzene as substrate. Acetaminophen and p-nitrophenol are used as substrates for **phenol sulfotransferase** in cytosol and for assessment of glucuronosyltransferase activity in microsomes. Raftogianis, R. B. et al., J. Pharmacol. Exp. Ther. 276:602-608 (1996); Habig, W. H. et al., J. Biol. Chem. 249:7130-7139 (1974) and Ritter, J. K. et al., Drug Metab. Dispos. 15:335-343 (1987). Sulfotransferase activity towards acetaminophen is determined in 0.1M citric acid-0.2M sodium phosphate buffer (pH 5.7) containing 5-10 mg/ml cytosolic protein, 500 .mu.M PAPS, and 600 .mu.M acetaminophen. The reaction is terminated by addition of perchloric acid after a 10 minute incubation at 25 C. Acetaminophen sulfate is measured by HPLC. Corcoran, G. B. et al., J. Pharmacol. Exp. Ther. 232:857-863 (1985). For determination of the glucuronidation of acetaminophen, the incubation mixture consists of 50 mM tris buffer (pH 7.85) containing 150 mM KCl, 10 mM MgCl.sub.2, 2 mg/mL microsomal protein, 60 mM acetaminophen and 0.05% Triton X-100. The reaction is initiated through addition of 5 mM UDPGA and terminated after 15 minutes at 37 C. by addition of perchloric acid.

US-PAT-NO: 6071732

DOCUMENT-IDENTIFIER: US 6071732 A

TITLE: Tyrosylprotein sulfotransferases, nucleic acids encoding tyrosylprotein sulfotransferases, and methods of use thereof

DATE-ISSUED: June 6, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

APPL-NO: 09/ 150141

DATE FILED: September 9, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application claims the benefit of U.S. Provisional Application Ser. No. 60/072,994, filed Jan. 29, 1998.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Some aspects of

this invention were made in the course of Grant AI 28018 awarded by the National Institutes of Health and therefore the Government has certain rights in some aspects of this invention.

US-CL-CURRENT: 435/193

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and *C. elegans*. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system.

3 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

----- KWIC -----

Detailed Description Text - DETX:

TPST-1 and other TPSTs exhibit homology to a large family of cytosolic **sulfotransferases, including phenol- and hydroxysteroid-sulfotransferases**. The known members of this family contain two regions which are highly conserved throughout phlogeny, called region I and region IV (31). These regions are involved in binding of the sulfate donor PAPS (32-35). Alignment of mouse TPST-1 and mouse estrogen sulfotransferase reveals a 20% identity and 52% similarity with 19 alignment gaps, 12 of which are 3 residues in length (FIG. 6). Notably, TPST-1 has a 35 residue amino terminal extension that includes the putative non-cleavable signal peptide/membrane anchor. In the estrogen sulfotransferase crystal structure, the residues which contact the 5' phosphate of PAPS (PKSGTTW (SEQ ID NO:44)) form a loop between .beta.-sheet 3 and .alpha.-helix 3, which corresponds to region I (35). This region is highly conserved in TPST-1, TPST-2, TPST-A, and TPST-B, and corresponds to residues 78-84 (PRSGTTL ((SEQ ID NO:45)) in TPST-1, residues 77-83 in TPST-2, residues 78-84 in TPST-A, and residues 94-100 in TPST-B (see FIG. 13). The residues involved in binding the 3' phosphate of PAP are located in two discontinuous regions of estrogen sulfotransferase. The first region includes two residues, Arg.sup.130 and Ser.sup.138, located just before and within .alpha.-helix 6. The second is comprised of residues 257-259 (Arg-Lys-Gly). The corresponding residues in TPST-1 are Arg.sup.184, Ser.sup.192, Ala.sup.322, Lys.sup.323, and Leu.sup.324. Thus, although the degree of identity is limited, most of the residues involved in PAPS binding in the estrogen sulfotransferase (SEQ ID NO:13) structure are predicted to be conserved in TPST (FIG. 6). TPST exhibits a similar degree of homology to Golgi sulfotransferases, including heparan sulfate 2-sulfotransferase (36), chondroitin 6-sulfotransferase (37), and the C-terminal domain of heparan sulfate N-deacetylase/N-sulfotransferase (38).

US-PAT-NO: 6060295

DOCUMENT-IDENTIFIER: US 6060295 A

TITLE: Nucleic acids and expression systems encoding tyrosylprotein sulfo transferases

DATE-ISSUED: May 9, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moore; Kevin L.	Oklahoma City	OK	N/A	N/A

APPL-NO: 09/ 150133

DATE FILED: September 9, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application claims the benefit of U.S. Provisional Application Ser. No. 60/072,994, filed Jan. 29, 1998.

US-CL-CURRENT: 435/193; 435/320.1 ; 435/325 ; 536/23.2

ABSTRACT:

Tyrosylprotein sulfotransferases and nucleic acids encoding the tyrosylprotein sulfotransferases are described. Dual isotopes of the enzyme and of the nucleic acids encoding said enzymes have been identified in human, mouse and C. elegans. The polypeptides and polynucleotides exhibit a wide range of homologies. The polynucleotides can be used to transform or transfect host cells for producing substantially pure forms of the enzyme, or for use in an expression system for post-translational tyrosine sulfation of proteins or peptides produced within the expression system.

29 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

----- KWIC -----

Detailed Description Text - DETX:

TPST-1 and other TPSTs exhibit homology to a large family of cytosolic sulfotransferases, including phenol- and hydroxysteroid-sulfotransferases. The known members of this family contain two regions which are highly conserved

throughout phlogeny, called region I and region IV (31). These regions are involved in binding of the sulfate donor PAPS (32-35). Alignment of mouse TPST-1 and mouse estrogen sulfotransferase reveals a 20% identity and 52% similarity with 19 alignment gaps, 12 of which are 3 residues in length (FIG. 6). Notably, TPST-1 has a 35 residue amino terminal extension that includes the putative non-cleavable signal peptide/membrane anchor. In the estrogen sulfotransferase crystal structure, the residues which contact the 5' phosphate of PAPS (PKSGTTW (SEQ ID NO:44)) form a loop between .beta.-sheet 3 and .alpha.-helix 3, which corresponds to region I (35). This region is highly conserved in TPST-1, TPST-2, TPST-A, and TPST-B, and corresponds to residues 78-84 (PRSGTTL (SEQ ID NO:45)) in TPST-1, residues 77-83 in TPST-2, residues 78-84 in TPST-A, and residues 94-100 in TPST-B (see FIG. 13). The residues involved in binding the 3' phosphate of PAP are located in two discontinuous regions of estrogen sulfotransferase. The first region includes two residues, Arg.sup.130 and ser.sup.138, located just before and within .alpha.-helix 6. The second is comprised of residues 257-259 (Arg-Lys-Gly). The corresponding residues in TPST-1 are Arg.sup.184, ser.sup.192, Ala.sup.322, Lys.sup.323, and Leu.sup.324. Thus, although the degree of identity is limited, most of the residues involved in PAPS binding in the estrogen sulfotransferase (SEQ ID NO:13) structure are predicted to be conserved in TPST (FIG. 6). TPST exhibits a similar degree of homology to Golgi sulfotransferases, including heparan sulfate 2-sulfotransferase (36), chondroitin 6-sulfotransferase (37), and the C-terminal domain of heparan sulfate N-deacetylase/N-sulfotransferase (38).

US-PAT-NO: 5744355

DOCUMENT-IDENTIFIER: US 5744355 A

TITLE: cDNA cloning and expression of human liver estrogen sulfotransferase

DATE-ISSUED: April 28, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weinshilbom; Richard	Rochester	MN	N/A	N/A
M.	Rochester	MN	N/A	N/A
Aksoy; Ibrahim A.	Rochester	MN	N/A	N/A
Wood; Thomas C.				

DISCLAIMER DATE: 20141018

APPL-NO: 08/ 437795

DATE FILED: May 9, 1995

PARENT-CASE:

This is a continuation-in-part application of U.S. patent application Ser. No. 08/325,562 filed Oct. 18, 1994, which is incorporated herein by reference.

US-CL-CURRENT: 435/325; 435/193 ; 435/252.3 ; 435/320.1 ; 536/23.1 ; 536/23.2

ABSTRACT:

The present invention provides an isolated and purified human DNA molecule that encodes human estrogen sulfotransferase.

8 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

TITLE - TI:

cDNA cloning and expression of human liver estrogen sulfotransferase

Abstract Text - ABTX:

The present invention provides an isolated and purified human DNA molecule that encodes human estrogen sulfotransferase.

Brief Summary Text - BSTX:

The metabolism of many drugs, xenobiotics, neurotransmitters and hormones includes a step involving the enzymatic addition of a sulfate (SO_4^{2-}) group. The addition of a sulfate group is commonly referred to as sulfate conjugation, or simply sulfation. The enzymes responsible for sulfate conjugation are known as sulfotransferases, as they act by transferring a sulfate group from one biological molecule (the sulfate donor) to another (the sulfate acceptor) in a sulfotransferase reaction.

Brief Summary Text - BSTX:

Cytosolic sulfotransferase (ST) enzymes in human liver have been subjected to intensive research. Sulfation increases the water solubility of most compounds and, therefore, their renal excretion. It also usually results in a decrease in biological activity. However, in some cases, sulfate conjugation is required to activate drugs such as the antihypertensive medication minoxidil (G. A. Johnson et al., Biochem. Pharmacol., 31, 2949-2954 (1982)), and it can also play a role in the bioactivation of procarcinogens such as hydroxylarylamines (T. Watabe et al., Science, 215, 403 (1982)).

Brief Summary Text - BSTX:

Human liver tissue is known to catalyze a number of sulfotransferase reactions, all of which utilize 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a sulfate donor (G. J. Mulder et al., Conjugation Reactions in Drug Metabolism, 107-161, Taylor & Francis Ltd., New York (1990)). Specific cytosolic sulfotransferase enzymes that are present in human liver include dehydroepiandrosterone sulfotransferase (DHEA ST) and two forms of phenol sulfotransferase (PST), known as thermolabile PST (TL PST) and thermostable PST (TS PST).

Brief Summary Text - BSTX:

These three enzymes can be characterized and classified by their thermostability, their sensitivity to inhibition by 2,6-dichloro-4-nitrophenol (DCNP), a competitive inhibitor of some types of sulfotransferase activity, and by their preferred substrates. DHEA ST catalyzes the sulfate conjugation of cholesterol, bile acids and steroid hormones. It is relatively thermostable and is relatively resistant to DCNP inhibition. TL PST is also relatively resistant to DCNP inhibition but is, as its name indicates, thermolabile. This sulfotransferase preferentially catalyzes the sulfate conjugation of micromolar concentrations of dopamine and other phenolic monoamines. In contrast, TS PST is thermostable, very sensitive to DCNP inhibition, and catalyzes the sulfation of micromolar concentrations of simple planar phenols such as 4-nitrophenol.

Brief Summary Text - BSTX:

Estrogens are not preferred substrates for any of these three human liver sulfotransferase enzymes. Only two (DHEA ST and TS PST) are even capable of catalyzing the sulfation of estrogens. Sulfotransferase enzymes specific for estrogen (estrogen sulfotransferases, known also as EST enzymes or simply "ESTs") are, however, known for several nonhuman species (A. R. Nash et al., Aust. J. Biol. Sci. 41, 507-516 (1988); W. F. Demyan et al., Mol. Endocrinol., 6, 589-597 (1992); T. Oeda et al., Mol. Endocrinol., 6 1216-1226 (1992)), and show a high degree of sequence homology. Pairwise comparisons between the amino acid sequences of ESTs in rat liver, bovine placenta and guinea pig adrenal cortex show a high level of identity (66-70%). This suggests that mammalian ESTs may be members of a subfamily within a sulfotransferase gene superfamily--a subfamily distinct from those to which the PSTs and DHEA ST belong. It has been suggested that differences in the formation of estrogen sulfates might play a role in variation in response to various estrogens and other structurally-related therapeutic agents (R. Hobkirk, Trends Endocrinol. Metab., 4, 69-74 (1993); A. K. Roy, Proc. Soc. Exp. Biol. Med., 199, 265-272 (1992); M. A. Mancini et al., Endocrinology, 131, 1541-1546 (1992)). It would be a significant medical advance to discover, clone, and characterize complementary DNA (cDNA) encoding a human EST enzyme.

Brief Summary Text - BSTX:

To date, vigorous genetic engineering efforts in the field of sulfate metabolism have resulted in the cloning and expression of cDNAs for several sulfotransferase enzymes: human liver DHEA ST (D. M. Otterness et al., Mol. Pharmacol., 41, 865-872 (1992)), TS PST (W. Wilborn et al., Mol. Pharmacol., 43, 70-77 (1993)), TL PST (T. C. Wood et al., Biochem. Biophys. Res. Commun., 198, 1119-1127 (1994)) and a group of ESTs isolated from several tissues of nonhuman mammalian species (A. R. Nash et al., Aust. J. Biol. Sci., 41, 507-516 (1988); W. F. Demyan et al., Mol. Endocrinol., 6, 589-597 (1992); T. Oeda et al., Mol. Endocrinol., 6, 1216-1226 (1992)). No group has yet identified or cloned a human estrogen sulfotransferase cDNA.

Brief Summary Text - BSTX:

Estrogen sulfotransferase activity (EST activity) has been detected in human liver (K. J. Forbes-Bamforth et al., Biochem. Biophys. Res. Commun., 198, 707-711 (1994)). However, a human EST cDNA was not isolated. Identification of a human estrogen sulfotransferase cDNA, and production of human estrogen sulfotransferase enzyme, would be useful in the determination of which endogenous steroid hormones and/or drugs might be metabolized by the protein encoded by the cDNA. Cell lines expressing a human EST enzyme could be constructed from the cloned EST cDNA using methods of genetic engineering known in the art. The resulting availability of recombinant human EST enzyme would be extremely useful to workers in the field of human reproductive metabolism. For example, the expressed human EST enzyme could be used in a laboratory

setting to identify other compounds, structurally related to estrogen, that are metabolized by human EST. The affinity of human EST enzyme for these substrates could be directly measured. Potential metabolic pathways of new therapeutic hormones developed by drug companies could be more easily predicted, and potential differences in their pharmacokinetic parameters could be anticipated prior to testing in humans. If human EST enzyme were available, experimental use of animals to study the metabolism of newly developed estrogen-like compounds would likely decrease, since animal ESTs will differ in their affinity for such compounds.

Brief Summary Text - BSTX:

Human EST enzyme is undoubtedly an important hormone and drug-metabolizing enzyme. It would be of significant clinical, diagnostic, and therapeutic importance to know which human tissues express an EST, and to have a means of quantifying that expression level. If a human cDNA sequence were known, tissue-specific expression of the human mRNA encoding EST could be investigated using Northern blot analysis. Likewise, tissue-specific EST protein expression could be studied. Identification of a human EST cDNA would lead directly to knowledge of the amino acid sequence encoded thereby. Synthetic antigens derived from this amino acid sequence could be used to obtain anti-EST antibodies useful for immunohistochemical location of EST enzyme in human tissues and for Western blot analysis of the protein expression level of EST in different human tissues. These techniques would not only permit determination of the extent of variation in the expression of EST in humans, but would also make possible the detection of mutations that may alter the ability of EST enzyme to catalyze the metabolism of endogenous hormones or of drugs that are structurally related to endogenous hormones. Therefore, what is needed is a DNA molecule that encodes human estrogen sulfotransferase.

Drawing Description Text - DRTX:

FIG. 3A. Sequence identity among various known cloned sulfotransferases. The enzymes compared include human liver EST (hEST), guinea pig adrenocortical EST (gpEST), bovine placental EST (bEST), rat liver EST (rEST), rat liver PST (rPST), mouse liver PST (mPST), human liver TS PST (hTSPST), rat liver hydroxysteroid ST (rHSST1 and rHSST2), rat liver senescence marker protein 2 (rSMP2), mouse hydroxysteroid (mHSST), human liver DHEA ST (hDHEAST), and Flaveria chloraefolia flavonol 3-ST and 4'-ST (fcFST3 and fcFST4'). Values shown represent the percent identity of amino acid sequences as determined by use of the BESTFIT program in the Genetics Computer Group (GCG) package (Madison, Wis.). Boxed values represent comparisons that show greater than 60% sequence identity.

Drawing Description Text - DRTX:

FIG. 3B. Dendrogram relating various known cloned sulfotransferases. The functional groupings indicated on the right represent sequences in (FIG. 3A) that were greater than 60% identical. Amino acid sequences were clustered by use of the PILEUP program in the GCG package (Madison, Wis.).

Drawing Description Text - DRTX:

The present invention provides an isolated and purified DNA encoding human estrogen sulfotransferase that hybridizes to DNA complementary to DNA having SEQ ID NO:1 under the stringency conditions of hybridization in buffer containing 20% formamide, 5.times. Denhardt's, 6.times. SSC, 100 mg/ml RNA and 0.05% sodium pyrophosphate, at 42.degree. C., followed by washing at 60.degree. C. and 1.times. SSC, 0.1% SDS. Preferably, the present invention provides an isolated and purified DNA encoding the estrogen sulfotransferase having an amino acid sequence which has the amino acid sequence shown in FIG. 2 (SEQ ID NO:2). Preferably, the DNA is cDNA which has the nucleotide sequence shown in FIG. 2 (SEQ ID NO: 1).

Drawing Description Text - DRTX:

Specifically, the present invention is directed to the cloning and expression of human estrogen sulfotransferase cDNA as well as the characterization and production of a human estrogen sulfotransferase enzyme (EST). To that end, the invention provides an isolated and purified human DNA encoding a human estrogen sulfotransferase (EST) protein or biologically active derivative thereof. More preferably, the cDNA molecule encodes the protein represented by the amino acid sequence shown in FIG. 2 (SEQ ID NO:2). Most preferably, the cDNA molecule is represented by the complete nucleotide sequence shown in FIG. 2 (SEQ ID NO:1). Isolated and purified peptides encoded by this DNA which are biologically active are also within the scope of the invention.

Drawing Description Text - DRTX:

As used herein, the terms "isolated and purified" refer to in vitro isolation of a DNA molecule or peptide from its natural cellular environment, and from association with other coding regions of the human genome, so that it can be sequenced, replicated, and/or expressed. Preferably, the isolated and purified DNA molecules of the invention comprise a single coding region. Thus, the present DNA molecules are those "consisting essentially of" a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof. Although the DNA molecule includes a single coding region, it can contain additional nucleotides that do not detrimentally affect the function of the DNA molecule, i.e., the expression of the estrogen sulfotransferase protein or biologically active derivative thereof. For example, the 5' and 3' untranslated regions may contain variable numbers of nucleotides. Preferably, additional nucleotides are outside the single coding region.

Drawing Description Text - DRTX:

The present invention also provides an isolated and purified DNA molecule that encodes human estrogen sulfotransferase protein and that hybridizes to a DNA molecule complementary to the DNA molecule shown in FIG. 2 (SEQ ID NO:1) under high stringency hybridization conditions. As used herein, "high stringency

hybridization conditions" refers to hybridization in buffer containing 20% formamide, 5.times. Denhardt's, 6.times. SSC, 100 mg/ml RNA, and 0.05% sodium pyrophosphate at 42.degree. C., followed by washing at 60.degree. C., 0.1% sodium dodecyl sulfate (SDS), and 1.times. SSC (1.times. SSC contains 0.15M sodium chloride and 0.015M trisodium citrate, pH 7.0).

Drawing Description Text - DRTX:

As used herein, the terms estrogen sulfotransferase protein, estrogen sulfotransferase enzyme, estrogen sulfotransferase (EST), and EST enzyme, are used interchangeably, and refer to a sulfotransferase enzyme that shows preference for estrone as a sulfation substrate over the other common sulfotransferase substrates, DHEA, 4-nitrophenol, or dopamine. A "biologically active derivative thereof" is a human estrogen sulfotransferase that is modified by amino acid deletion, addition, substitution, or truncation, or that has been chemically derivatized, but that nonetheless utilizes estrogen as its preferred sulfate-acceptor substrate, and sulfates estrone at a higher level than it sulfates DHEA, 4-nitrophenol, or dopamine. For example, it is known in the art that substitutions of aliphatic amino acids such as alanine, valine and isoleucine with other aliphatic amino acids can often be made without altering the structure or function of a protein. Similarly, substitution of aspartic acid for glutamic acid, in regions other than the active site of an enzyme, are likely to have no appreciable effect on protein structure or function. The term "biologically active derivative" is intended to include ESTs as thus modified. The term also includes fragments, variants, analogs or chemical derivatives of human EST enzyme. The term "fragment" is meant to refer to any polypeptide subset of human EST enzyme. Fragments can be prepared by subjecting human EST to the action of any one of a number of commonly available proteases, such as trypsin, chymotrypsin or pepsin, or to chemical cleavage agents, such as cyanogen bromide. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire human EST molecule or to a fragment thereof. A molecule is said to be "substantially similar" to human EST or a fragment thereof if both molecules have substantially similar amino acid sequences, preferably greater than about 80% sequence identity, or if the three-dimensional backbone structures of the molecules are superimposable, regardless of the level of identity between the amino acid sequences. Thus, provided that two molecules possess estrogen sulfotransferase activity, they are considered variants as that term is used herein even if the structure of one of the molecules is not found in the other, or if the sequences of amino acid residues are not identical. The term "analog" is meant to refer to a protein that differs structurally from the wild type enzyme EST, but possesses sulfotransferase activity utilizing estrogen as a preferred substrate.

Drawing Description Text - DRTX:

The present invention also provides a vector comprising an isolated and purified DNA molecule encoding human estrogen sulfotransferase or a biologically active derivative thereof, preferably the sulfotransferase having the amino acid sequence of FIG. 2. Preferably, the vector includes a single

estrogen sulfotransferase coding region as well as a second DNA segment operably linked to the coding sequence and capable of directing expression of human estrogen sulfotransferase, such as a promoter region operably linked to the 5' end of the coding DNA sequence. The vector can also include a DNA segment that is a selectable marker gene or a reporter gene.

Drawing Description Text - DRTX:

The present invention also provides a cell line, preferably mammalian, the genome of which has been augmented by chromosomally integrated non-native DNA encoding human estrogen sulfotransferase as herein described.

Drawing Description Text - DRTX:

In a preferred embodiment of the invention, the eukaryotic host cell COS-1 is transformed with the eukaryotic expression vector p91023(B), into which a cDNA molecule encoding human estrogen sulfotransferase has been subcloned. The transformed COS-1 host cells of the invention are grown, expression of estrogen sulfotransferase is induced, and the cells are harvested and processed using methods and procedures well-known in the art. This genetically engineered expression system provided by the invention is thus a convenient source of human estrogen sulfotransferase.

Drawing Description Text - DRTX:

Sulfotransferases may be purified using anion exchange chromatography, typically performed with DEAE-cellulose or DEAE Sepharose CL-6B, to separate, at least partially, different ST activities. Other chromatographic techniques that may be used in the purification of ST enzymes include hydroxylapatite and gel filtration, preferably in combination with one or more of a variety of affinity chromatographic columns with varying degrees of specificity for ST enzymes. Affinity columns that may be used include Affi-Gel Blue, ATP-agarose chromatography, heparin-Sepharose, ADP-agarose, PAP-agarose, estradiol-17.β.-Sepharose, and p-hydroxyphenylacetic acid-agarose. The availability of purified EST enzyme makes it possible to characterize the enzyme and to develop antibodies that can be used, for example, to screen cDNA expression libraries.

Drawing Description Text - DRTX:

General sulfotransferase activity may be assayed utilizing radioactively-labeled 3'-phosphoadenosine-5'-phosphosulfate (PAPS) (e.g., .sup.35 S-PAPS) or of non-radioactive PAPS with a radioisotope labeled sulfate acceptor substrate. Commonly, a barium precipitation assay is used, in which .sup.35 S-PAPS and .sup.35 SO₄²⁻ are precipitated by barium (A. Foldes et al., Biochim. Biophys. Acta, 327 365 (1973); R. J. Anderson et al., Clin. Chim. Acta, 103, 79 (1980)). Barium salts of most sulfated reaction products are soluble in water, i.e., they are not precipitated. Specifically, these assays involve incubation of a sulfate acceptor substrate with .sup.35

S-PAPS, followed by termination of the enzymatic reaction by the addition of Ba(OH)₂ and ZnSO₄. This step is followed by the removal of precipitated ³⁵S-PAPS by centrifugation, while the radioactively-labeled sulfated product remains in the aqueous phase. Radioactivity in the supernatant is then measured in a liquid scintillation counter. Alternatively, ³⁵S-PAPS can be separated from sulfated reaction products using thin layer chromatography (R. D. Sekura et al., Anal. Biochem. 95 82 (1979)), Ecteola cellulose chromatographic columns (R. T. Borchardt et al., Anal. Biochem., 130, 334 (1983); R. M. Whittemore et al., Biochem. Pharmacol., 34, 1647 (1985)), high performance liquid chromatography (T. Honkasalo et al., J. Chromatogr., 424, 136 (1988), or organic solvent extraction (L. Varin et al., Anal. Biochem., 161, 176 (1987).

Drawing Description Text - DRTX:

Estrogen sulfotransferase activity can be detected and quantified using an enzymatic assay provided by the invention. This assay can be used to measure EST activity in biological samples, preferably human samples, more preferably human liver preparations. The assay is extremely sensitive because it makes use of very highly radioactively labeled ³⁵S-PAPS; depending upon the expression of the protein in a given tissue, the assay is as sensitive as a radioimmunoassay. It can be used to detect the estrogen sulfotransferase activity of any sulfotransferase enzyme, for example, human EST, DHEA ST and TS PST. The substrate used in the assay can be any estrogen or related chemical compound. Preferably, the substrate is estrone, estradiol-17 β , ethinyl estradiol or dehydroepiandrosterone (DHEA). More preferably, estrone is used as the sulfate acceptor substrate in the assay. The assay contains estrone and a magnesium (Mg²⁺) salt in a potassium phosphate buffer at a pH of about 8 to 9. As optimized for assaying human liver extracts, the reaction mixture used in the assay contains 0.3 mM MgCl₂, 25 μ M estrone, and 50 mM potassium phosphate, pH 8.25. When specifically optimized for assaying EST activity of human EST enzyme expressed in crude extracts from the recombinant EST-P91023(B) COS-1 constructs, described herein, the reaction mixture contains 0.039-10 (preferably 1.25) mM MgCl₂, 0.05 μ M estrone and 8.2 mM potassium phosphate, pH 5.0-8.9 (preferably 6.5).

Drawing Description Text - DRTX:

Isolation and expression of a cDNA for human liver EST, and the development of an assay for EST activity, represent important steps toward understanding the biotransformation of estrogens in humans. The EST activity assay, while very sensitive, is not, however, specific for the human EST enzyme, since other known sulfotransferases also exhibit varying levels of EST activity. Specific identification of human EST enzyme and quantification of its expression level is nonetheless possible through use of the human EST cDNA provided by the invention. Knowledge of the human EST cDNA sequence leads to knowledge of the EST protein sequence, which in turn makes possible the production of antibodies against portions of the protein sequence specific to human EST protein. Western blot analysis utilizing such antibodies, and Northern analysis using human EST cRNA, are likely to provide highly specific assays for human EST protein expression.

Detailed Description Text - DETX:

The sequence of the protein encoded by this human liver EST cDNA was 81, 73 and 72% identical with the amino acid sequences of guinea pig adrenocortical, bovine placental, and rat liver ESTs, respectively (FIG. 3A) (A. R. Nash et al., *Aust. J. Biol. Sci.*, 41, 507-516 (1988); W. F. Demyan et al., *Mol. Endocrinol.*, 6, 589-597 (1992); T. Oeda et al., *Mol. Endocrinol.*, 6, 1216-1226 (1992)). However, it was only 37, 51 and 51% identical with the deduced amino acid sequences of human liver DHEA ST, TS **PST** and TL **PST**, respectively (FIG. 3A) (D. M. Otterness et al., *Mol. Pharmacol.*, 41, 865-872 (1992); T. W. Wilborn et al., *Mol. Pharmacol.*, 43, 70-77 (1993); T. C. Wood et al., *Biochem. Biophys. Res. Commun.*, 198, 1119-1127 (1994)). Comparison of the deduced amino acid sequence of EST with those of 13 other ST enzymes showed many areas of sequence homology, two of which have been observed to be highly conserved throughout phylogeny. One of those sequences, YPKSGTXW (SEQ ID NO: 16), is located toward the amino, and the other, RKGXXGDWKNXFT (SEQ ID NO: 17), toward the carboxy terminus of the proteins. As used in the amino acid sequences disclosed herein, "X" indicates any amino acid, and the other letters identify specific amino acids in accordance with the standard single-letter code used in the art. Comparisons of percentages of amino acid sequence identities (FIG. 3A) and a dendrogram depicting graphically the relationships among these proteins (FIG. 3B) confirmed that the STs are a gene superfamily with striking sequence identity among orthologous enzymes across species lines.

Detailed Description Text - DETX:

The insert subcloned into pBluescript (Example I) contained the entire coding region of the human liver EST cDNA. The major in vitro translation product had an apparent relative molecular weight (M.sub.r) of 34.6 kDa as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The M.sub.r of recombinant human liver EST enzyme, calculated on the basis of amino acid sequence deduced from the sequence of the cDNA, was 35.1 kDa. ST activities were measured in preparations of transfected COS- 1 cells using DHEA, 4- nitrophenol and dopamine, model substrates for DHEA ST, TS **PST** and TL **PST**, respectively (Example IV). The assays were performed under optimal conditions for the measurement of these three enzyme activities in human liver preparations (N. R. C. Campbell et al., *Biochem. Pharmacol.*, 36, 1435-1446 (1987); J. S. Hernandez et al., *Drug Metab. Dispos.*, 20, 413-422 (1992), both of which are incorporated herein by reference). The sulfation of estrone was measured using optimal conditions for the assay of EST activity of human EST enzyme (Example III(c)). The protein encoded by the human liver EST cDNA was capable of catalyzing the sulfation of estrone (.about.9 units/mg protein), DHEA (.about.2 units/mg protein) and 4.about.nitrophenol (.about.5 units/mg protein), but not that of dopamine (Table 2). When transfection was performed with p91023(B) alone or with only buffer, no detectable ST activity was present with any of the substrates tested.

Detailed Description Text - DETX:

Development of an Estrogen Sulfotransferase Activity Assay

Detailed Description Text - DETX:

A. Optimized Estrogen Sulfotransferase Activity Assay for Human

Detailed Description Text - DETX:

The ability of the sulfotransferases present in human liver to catalyze the sulfation of estrone (i.e., the EST activity of the sulfotransferases) was assayed using a dilute sample of HSS (high speed supernatant) obtained from crude human liver extracts. Pre-diluted HSS was first made by diluting 50 .mu.l HSS into 450 .mu.l dilution buffer (dilution buffer: 5 mM potassium phosphate, pH 6.5, containing 1.5 mg/ml bovine serum albumin and 1.54 mg/ml dithiothreitol). Diluted HSS was then prepared by diluting 210 .mu.l of the pre-diluted HSS into 6790 .mu.l of dilution buffer, such that 100 .mu.l dilute HSS=0.3 .mu.l HSS, and kept on ice; assays used a 100 .mu.l aliquot of diluted HSS. Concentrated estrone substrate solution was prepared by dissolving estrone in dimethylsulfoxide (DMSO) (0.215 mg estrone/ml DMSO). Concentrated MgCl.sub.2.6H.sub.2 O solution (1.95 mg MgCl.sub.2.6H.sub.2 O/ml H.sub.2 O) and concentrated DTT solution (7.4 mg/mL H.sub.2 O) were prepared for use in the optimized EST assay.

Detailed Description Text - DETX:

The recombinant protein encoded by the EST cDNA expressed in the COS-1 construct appeared to have greater sulfotransferase activity when estrone was the sulfate acceptor substrate than was the case with estradiol-17.beta., ethinyl estradiol, or DHEA.

Detailed Description Text - DETX:

C. Optimized Estrogen Sulfotransferase Activity Assay for Human Recombinant EST Enzyme.

Detailed Description Text - DETX:

A. Sulfotransferase (ST) activity.

Detailed Description Text - DETX:

The ability of human EST enzyme to catalyze sulfation of various substrates was compared. ST activities were assayed by the method of Foldes et al. (Biochim. Biophys. Acta, 327, 365-374 (1973)), as modified by Hernandez et al. (Drug Metab. Dispos. 20, 413-422 (1992)) for the measurement of human liver DHEA ST activity with its preferred substrate, DHEA (Sigma Chemical Co., St. Louis, Mo.), and by Campbell et al. (Biochem. Pharmacol., 36, 1435-1446 (1987)), for

the measurement of human liver TS and TL **PST** activities with their preferred substrates, 4-nitrophenol and dopamine, respectively (Sigma Chemical Co., St. Louis, Mo.). These assays were performed on high-speed supernatants (HSS) of crude COS-1 cell extracts under optimal conditions for the measurement of ST enzyme activities in human liver preparations as described in N. R. C. Campbell et al. (Biochem. Pharmacol., 36, 1435-1446 (1987)) and J. S. Hernandez et al. (Drug Metab. Dispos., 20, 413-422 (1992)), both of which are incorporated herein by reference. For the measurement of estrone sulfating activity, the optimized assay conditions for human EST enzymes described in Example III(c) were used. Specifically, the sulfate acceptor substrates used in the various assays were DHEA (5 .mu.M), 4-nitrophenol (4 .mu.M), dopamine (60 .mu.M), and estrone (0.05 .mu.M), respectively. Controls did not contain sulfate acceptor substrates. 1. DHEA ST activity of human EST enzyme.

Detailed Description Text - DETX:

2. TS **PST** activity of human EST enzyme.

Detailed Description Text - DETX:

3. TL **PST** activity of human EST enzyme.

Detailed Description Text - DETX:

The ability of human EST enzyme to catalyze the sulfation of dopamine was also assayed using a dilute sample of HSS. In this case, the dilution buffer described above was used to dilute HSS such that 100 .mu.l dilute HSS=0.1 .mu.l HSS. The assay was carried out substantially as described for the TS **PST** assay above, with the following exceptions. Five .mu.l of a concentrated solution of pargyline (1 mM final reaction concentration) was added to all tubes prior to the addition of dopamine. In place of 4-nitrophenol, 5 .mu.l of a concentrated dopamine solution was used as a substrate, such that the final reaction concentration was 60 .mu.M dopamine.

Detailed Description Text - DETX:

For all **sulfotransferase** assays, one unit of enzyme activity represented the formation of 1 nmol of sulfated product per hour of incubation at 37.degree. C. All assays were performed in triplicate, and values reported in Table 2 are averages of those three determinations. Protein concentrations were measured by the method of Bradford (Anal. Biochem., 72, 248-254 (1976)) with bovine serum albumin as a standard.

Claims Text - CLTX:

1. A cell line, the genome of which has been augmented by a chromosomally integrated DNA segment having a nucleotide sequence that encodes a human estrogen **sulfotransferase** having the amino acid sequence of SEQ ID NO:2 or a

biologically active fragment of said human estrogen sulfotransferase.

Claims Text - CLTX:

2. The cell line of claim 1 wherein the DNA segment encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

4. The cell line of claim 1 wherein the DNA segment encodes a biologically active fragment of a human estrogen sulfotransferase, wherein the human estrogen sulfotransferase has the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

5. A vector comprising a DNA segment having a nucleotide sequence that encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2 or a biologically active fragment of said human estrogen sulfotransferase.

Claims Text - CLTX:

6. The vector of claim 5 wherein the DNA segment encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

8. The vector of claim 5 wherein the DNA segment encodes a biologically active fragment of a human estrogen sulfotransferase, wherein the human estrogen sulfotransferase has the amino acid sequence of SEQ ID NO:2.

Other Reference Publication - OREF:

T. Hondoh et al., "Purification and Properties of Estrogen Sulfotransferase of Human Fetal Liver", Biomedical Research 14(2) 129-136, Apr. 1993.

Other Reference Publication - OREF:

I.A. Aksoy et al., "Cholesterol Sulfation in Human Liver: Catalysis by Dehydroepiandrosterone Sulfotransferase," Drug Metab. Dispos., 21, 268-276 (1993).

Other Reference Publication - OREF:

I.A. Aksoy et al., "Human liver dehydroepiandrosterone sulfotransferase: Nature

and extent of individual variation", Clin. Pharmacol. Ther., 54, 498-506 (1993).

Other Reference Publication - OREF:

I.A. Askoy, et al., "Human Liver Estrogen Sulfotransferase: Identification by cDNA Cloning and Expression", Biochem. and Biophys. Research Commun. 200 1621-1629 (May 16, 1994).

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Liver Estrogen Sulfotransferase (EST): Polymerase Chain Reaction (PCR) Cloning of cDNA", (Abstract) ISSX Proc., 4 181, 1993.

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Liver Estrogen Sulfotransferase (EST): Polymerase Chain Reaction (PCR) Cloning of cDNA", (Poster Presentation for the Fifth North American Meeting of the International Society for the Study of Xenobiotics) Oct. 18, 1993.

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Estrogen Sulfotransferase. cDNA Cloning, Expression and Characterization", (Abstract), Proc. Amer. Assoc. Cancer Res., 35, 276 (1994).

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Estrogen Sulfotransferase: cDNA Cloning, Expression, and Characterization", (Poster Presentation for the Eighty-Fifth Annual Meeting of the American Association for Cancer Research (AACR)) Apr. 9-13, 1994.

Other Reference Publication - OREF:

R.T. Borchardt, et al., "An Ecteola-Cellulose Chromatography Assay for 3'-Phosphoadenosine 5'-Phosphosulfate: Phenol Sulfotransferase," Anal. Biochem., 130, 334-338 (1983).

Other Reference Publication - OREF:

N.R.C. Campbell et al., "Human Liver Phenol Sulfotransferase: Assay Conditions, Biochemical Properties and Partial Purification of Isozymes of the Thermostable Form," Biochem. Pharmacol., 36, 1435-1446 (1987).

Other Reference Publication - OREF:

W.F. Demyan et al., "Estrogen Sulfotransferase of the Rat Liver: Complementary DNA Cloning and Age- and Sex-Specific Regulation of Messenger RNA," Mol. Endocrinol., 6, 589-597 (1992).

Other Reference Publication - OREF:

K.J. Forbes-Bamforth et al., "Identification of a New Adult Human Liver Sulfotransferase with Specificity for Endogenous and Xenobiotic Estrogens," Biochem. Biophys. Res. Comm., 198, 707-711 (1994).

Other Reference Publication - OREF:

J.S. Hernandez et al., "Sulfation of Estrone and 17.β-Estradiol in Human Liver: Catalysis by Thermostable Phenol Sulfotransferase and by Dehydroepiandrosterone Sulfotransferase," Drug. Metab. Dispos., 20, 413-422 (1992).

Other Reference Publication - OREF:

W.B. Jakoby et al., "Sulfotransferases" in Enzymatic Basis of Detoxication; vol. II, W.B. Jakoby (ed), Academic Press: New York; pp. 199-229 (1980).

Other Reference Publication - OREF:

G.A. Johnson et al., "Sulfation of Minoxidil by Liver Sulfotransferase," Biochem. Pharmacol., 31, 2949-2954 (1982).

Other Reference Publication - OREF:

A-N.T. Kong et al., "Molecular Cloning of the Alcohol/Hydroxysteroid Form (mST.sub.al) of Sulfotransferase from Mouse Liver," Pharmaceut. Res., 10, 627-630 (1993).

Other Reference Publication - OREF:

A-N.T. Kong et al., "Molecular Cloning of cDNA Encoding the Phenol/Aryl Form of Sulfotransferase (mST.sub.pl) from Mouse Liver," Biochem. Biophys. Acta, 1171, 315-318 (1993).

Other Reference Publication - OREF:

M.A. Mancini et al., "Spatio-Temporal Expression of Estrogen Sulfotransferase within the Hepatic Lobule of Male Rats: Implication of in Situ Estrogen

Inactivation in Androgen Action," Endocrinology, 131, 1541-1546 (1992).

Other Reference Publication - OREF:

A.R. Nash et al., "Oestrogen **Sulfotransferase**: Molecular Cloning and Sequencing of cDNA for Bovine Placental Enzyme," Aust. J. Biol. Sci., 41, 507-516 (1988).

Other Reference Publication - OREF:

T. Oeda et al., "Molecular Cloning and Expression of a Full-Length Complementary DNA Encoding the Guinea Pig Adrenocortical Estrogen **Sulfotransferase**," Mol. Endocrinol., 6, 1216-1226 (1992).

Other Reference Publication - OREF:

D.M. Otterness et al., "Human Liver Dehydroepiandrosterone **Sulfotransferase**: Molecular Cloning and Expression of cDNA," Mol. Pharmacol., 41, 865-872 (1992).

Other Reference Publication - OREF:

D.M. Otterness et al., "Human Liver Thermostable **Phenol Sulfotransferase**: Photoaffinity Labeling with 2-Iodo-4-Azidophenol," Mol. Pharmacol., 36, 856-865 (1989).

Other Reference Publication - OREF:

R.A. Price et al., "Genetic Polymorphism for Human Platelet Thermostable **Phenol Sulfotransferase** (TS **PST**) Activity," Genetics, 122, 905-914 (1989).

Other Reference Publication - OREF:

C. Reiter et al., "Thermolabile and Thermostable Human Platelet **Phenol Sulfotransferase**: Substrate Specificity and Physical Separation," Naunyn-Schmied. Archiv. Pharmacol., 324, 140-147 (1983).

Other Reference Publication - OREF:

R.D. Sekura et al., "Assay of **Sulfotransferases**," Analyt. Biochem., 95, 82-86 (1979).

Other Reference Publication - OREF:

R.S. Sundaram et al., "Human Intestinal **Phenol Sulfotransferase**: Assay Conditions, Activity Levels and Partial Purification of the Thermolabile Form,"

Drug Metab. Dispos., 17, 255-264 (1989).

Other Reference Publication - OREF:

L. Tseng et al., "Estrogen Sulfotransferase in Human Placenta," J. Steroid Biochem., 22, 611-615 (1985).

Other Reference Publication - OREF:

L. Varin et al., "Enzymatic Assay for Flavonoid Sulfotransferase," Anal. Biochem., 161, 176-180 (1987).

Other Reference Publication - OREF:

L. Varin et al., "Molecular Characterization of Two Plant Flavonol Sulfotransferase," Proc. Natl. Acad. Sci. USA, 89, 1286-1290 (1992).

Other Reference Publication - OREF:

R.M. Weinshilboum, "Sulfotransferase Pharmacogenetics," Pharmacol. Ther., 45, 93-107 (1990).

Other Reference Publication - OREF:

R.M. Weinshilboum et al., "Sulfotransferase Enzymes," in Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity; Handbook of Experimental Pharmacology series; F.C. Kauffman, Ed.; Springer-Verlag (1994).

Other Reference Publication - OREF:

R.M. Whittemore et al., "A Modified Ecteola Cellulose Assay for M and P Phenol Sulfotransferase," Biochem. Pharmacol., 34, 1647-1652 (1985).

Other Reference Publication - OREF:

T.W. Wilborn et al., "Sequence Analysis and Expression of the cDNA for the Phenol-Sulfating Form of Human Liver Phenol Sulfotransferase," Mol. Pharmacol., 43, 70-77 (1993).

Other Reference Publication - OREF:

T.C. Wood et al., "Human Liver Thermolabile Phenol Sulfotransferase: cDNA Cloning, Expression and Characterization," Biochem. Biophys. Res. Commun., 198,

1119-1127 (1994).

US-PAT-NO: 5714594

DOCUMENT-IDENTIFIER: US 5714594 A

TITLE: cDNA cloning and expression of human liver estrogen sulfotransferase

DATE-ISSUED: February 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weinshilboum; Richard M.	Rochester	MN	N/A	N/A
Aksoy; Ibrahim A.	Rochester	MN	N/A	N/A
Wood; Thomas C.				

APPL-NO: 08/ 325562

DATE FILED: October 18, 1994

US-CL-CURRENT: 536/23.2; 435/193 ; 435/252.3 ; 435/320.1 ; 536/23.1

ABSTRACT:

The present invention provides an isolated and purified human DNA molecule consisting essentially of a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof.

9 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

TITLE - TI:

cDNA cloning and expression of human liver estrogen sulfotransferase

Abstract Text - ABTX:

The present invention provides an isolated and purified human DNA molecule consisting essentially of a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof.

Brief Summary Text - BSTX:

The metabolism of many drugs, xenobiotics, neurotransmitters and hormones includes a step involving the enzymatic addition of a sulfate (SO₄²⁻) group. The addition of a sulfate group is commonly referred to as sulfate conjugation, or simply sulfation. The enzymes responsible for sulfate conjugation are known as sulfotransferases, as they act by transferring a sulfate group from one biological molecule (the sulfate donor) to another (the sulfate acceptor) in a sulfotransferase reaction.

Brief Summary Text - BSTX:

Human liver tissue is known to catalyze a number of sulfotransferase reactions, all of which utilize 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a sulfate donor (G. J. Mulder et al., Conjugation Reactions in Drug Metabolism, 107-161, Taylor & Francis Ltd., New York (1990)). Specific cytosolic sulfotransferase enzymes that are present in human liver include dehydroepiandrosterone sulfotransferase (DHEA ST) and two forms of phenol sulfotransferase (PST), known as thermolabile PST (TL PST) and thermostable PST (TS PST).

Brief Summary Text - BSTX:

These three enzymes can be characterized and classified by their thermostability, their sensitivity to inhibition by 2,6-dichloro-4-nitrophenol (DCNP), a competitive inhibitor of some types of sulfotransferase activity, and by their preferred substrates. DHEA ST catalyzes the sulfate conjugation of cholesterol, bile acids and steroid hormones. It is relatively thermostable and is resistant to DCNP inhibition. TL PST is also resistant to DCNP inhibition but is, as its name indicates, thermolabile. This sulfotransferase preferentially catalyzes the sulfate conjugation of micromolar concentrations of dopamine and other phenolic monoamines. In contrast, TS PST is thermostable, sensitive to DCNP inhibition, and catalyzes the sulfation of micromolar concentrations of simple planar phenols such as 4-nitrophenol.

Brief Summary Text - BSTX:

Estrogens are not preferred substrates for any of these three human liver sulfotransferases. Only two (DHEA ST and TS PST) are even capable of catalyzing the sulfation of estrogens. Sulfotransferases specific for estrogen (estrogen sulfotransferases, or "ESTs") are, however, known for several nonhuman species (A. R. Nash et al., Aust. J. Biol. Sci., 41, 507-516 (1988); W. F. Demyan et al., Mol. Endocrinol., 6, 589-597 (1992); T. Oeda et al., Mol. Endocrinol., 6, 1216-1226 (1992)), and show a high degree of sequence homology. Pairwise comparisons between the amino acid sequences of ESTs in rat liver, bovine placenta and guinea pig adrenal cortex show a high level of identity (66-70%). This suggests that mammalian ESTs may be members of a subfamily within a sulfotransferase gene superfamily--a subfamily distinct from those to which the PSTs and DHEA ST belong. It has been suggested that differences in the formation of estrogen sulfates might play a role in variation in response to various estrogens and other structurally-related therapeutic agents (R.

Hobkirk, Trends Endocrinol. Metab., 4, 69-74 (1993); A. K. Roy, Proc. Soc. Exp. Biol. Med., 199, 265-272 (1992); M. A. Mancini et al., Endocrinology, 131, 1541-1546 (1992)). It would be a significant medical advance to discover, clone, and characterize a human EST complementary DNA (cDNA).

Brief Summary Text - BSTX:

To date, vigorous genetic engineering efforts in the field of sulfate metabolism have resulted in the cloning and expression of cDNAs for several sulfotransferases: human liver DHEA ST (D. M. Otterness et al., Mol. Pharmacol., 41, 865-872 (1992)), TS PST (W. Wilborn et al., Mol. Pharmacol., 43, 70-77 (1993)), TL PST (T. C. Wood et al., Biochem. Biophys. Res. Commun., 198, 1119-1127 (1994)) and a group of ESTs isolated from several tissues of nonhuman mammalian species (A. R. Nash et al., Aust. J. Biol. Sci., 41, 507-516 (1988); W. F. Demyan et al., Mol. Endocrinol., 6, 589-597 (1992); T. Oeda et al., Mol. Endocrinol., 6, 1216-1226 (1992)). No group has yet identified or cloned a human estrogen sulfotransferase cDNA.

Brief Summary Text - BSTX:

Estrogen sulfotransferase activity has been detected in human liver (K. J. Forbes-Bamforth et al., Biochem. Biophys. Res. Commun., 198, 707-711 (1994)). However, a human EST cDNA was not isolated. Identification of a human estrogen sulfotransferase cDNA, and production of human estrogen sulfotransferase, would be useful in the determination of which endogenous steroid hormones and/or drugs might be metabolized by the protein encoded by the cDNA. Therefore, what is needed is a DNA molecule that encodes human estrogen sulfotransferase.

Drawing Description Text - DRTX:

FIGS. 3 and 3B. A. Sequence identity among various known sulfotransferases. B. Dendrogram relating various known sulfotransferases. The University of Wisconsin Genetics Computer Group software package was used to analyze sequence information and to make the comparisons among the sequences of ST cDNA.

Detailed Description Text - DETX:

The present invention provides an isolated and purified human DNA molecule consisting essentially of a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof. Preferably, the DNA molecule is cDNA. In particularly preferred embodiments, the protein is represented by the amino acid sequence SEQ ID NO:2 shown in FIG. 2 and the DNA molecule is represented by the nucleotide sequence SEQ ID NO:1 shown in FIG. 2.

Detailed Description Text - DETX:

Specifically, the present invention is directed to the cloning and expression

of human estrogen sulfotransferase cDNA as well as the characterization and production of a human estrogen sulfotransferase (EST). To that end, the invention provides an isolated and purified human DNA molecule consisting essentially of a DNA segment encoding a human estrogen sulfotransferase (EST) protein or biologically active derivative thereof. More preferably, the cDNA molecule encodes the protein represented by the amino acid sequence SEQ. ID NO:2 shown in FIG. 2. Most preferably, the cDNA molecule is represented by the complete nucleotide sequence SEQ ID NO:1 shown in FIG. 2. Isolated and purified peptides encoded by this DNA which are biologically active are also within the scope of the invention.

Detailed Description Text - DETX:

As used herein, the terms "isolated and purified" refer to in vitro isolation of a DNA molecule or peptide from its natural cellular environment, and from association with other coding regions of the human genome, so that it can be sequenced, replicated, and/or expressed. Preferably, the isolated and purified DNA molecules of the invention comprise a single coding region. Thus, the present DNA molecules are those "consisting essentially of" a DNA segment encoding an estrogen sulfotransferase protein or biologically active derivative thereof. Although the DNA molecule includes a single coding region, it can contain additional nucleotides that do not detrimentally affect the function of the DNA molecule, i.e., the expression of the estrogen sulfotransferase protein or biologically active derivative thereof. For example, the 5' and 3' untranslated regions may contain variable numbers of nucleotides. Preferably, additional nucleotides are outside the single coding region.

Detailed Description Text - DETX:

The present invention also provides an isolated and purified DNA molecule that encodes an estrogen sulfotransferase protein and that hybridizes to a DNA molecule complementary to the DNA molecule shown in FIG. 2 under high stringency hybridization conditions. As used herein, "high stringency hybridization conditions" refers to at least about 60.degree. C., 0.1% SDS, 0.5.times.SSC.

Detailed Description Text - DETX:

As used herein, the term "estrogen sulfotransferase protein" refers to a sulfotransferase enzyme that shows preference for estrone as a sulfation substrate over the other common sulfotransferase substrates, DHEA, 4-nitrophenol, or dopamine. A "biologically active derivative thereof" is a human estrogen sulfotransferase that is modified by amino acid deletion, addition, substitution, or truncation, or that has been chemically derivatized, but that nonetheless utilizes estrogen as its preferred sulfate-acceptor substrate, and sulfates estrone at a higher level than it sulfates DHEA, 4-nitrophenol, or dopamine. For example, it is known in the art that substitutions of aliphatic amino acids such as alanine, valine and isoleucine with other aliphatic amino acids can often be made without altering the

structure or function of a protein. Similarly, substitution of aspartic acid for glutamic acid, in regions other than the active site of an enzyme, are likely to have no appreciable affect on protein structure or function. The term "biologically active derivative" is intended to include ESTs as thus modified. The term also includes fragments, variants, analogs or chemical derivatives of human EST. The term "fragment" is meant to refer to any polypeptide subset of human EST. Fragments can be prepared by subjecting human EST to the action of any one of a number of commonly available proteases, such as trypsin, chymotrypsin or pepsin, or to chemical cleavage agents, such as cyanogen bromide. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire human EST molecule or to a fragment thereof. A molecule is said to be "substantially similar" to EST or a fragment thereof if both molecules have substantially similar amino acid sequences, preferably greater than about 80% sequence identity, or if the three-dimensional backbone structures of the molecules are superimposable, regardless of the level of identity between the amino acid sequences. Thus, provided that two molecules possess estrogen sulfotransferase activity, they are considered variants as that term is used herein even if the structure of one of the molecules is not found in the other, or if the sequences of amino acid residues are not identical. The term "analog" is meant to refer to a protein that differs structurally from the wild type enzyme EST, but possesses sulfotransferase activity utilizing estrogen as a preferred substrate.

Detailed Description Text - DETX:

The present invention also provides a vector comprising an isolated and purified DNA molecule encoding human estrogen sulfotransferase or a biologically active derivative thereof, preferably the sulfotransferase having the amino acid sequence SEQ ID NO:2 of FIG. 2. Preferably, the vector includes a single estrogen sulfotransferase coding region as well as a second DNA segment operably linked to the coding sequence and capable of directing expression of human estrogen sulfotransferase, such as a promoter region operably linked to the 5' end of the coding DNA sequence. The vector can also include a DNA segment that is a selectable marker gene or a reporter gene.

Detailed Description Text - DETX:

In a preferred embodiment of the invention, the eukaryotic host cell COS-1 is transformed with the eukaryotic expression vector p91023(B), into which a cDNA molecule encoding human estrogen sulfotransferase has been subcloned. The transformed COS-1 host cells of the invention are grown, expression of estrogen sulfotransferase is induced, and the cells are harvested and processed using methods and procedures well-known in the art. This genetically engineered expression system provided by the invention is thus a convenient source of human estrogen sulfotransferase.

Detailed Description Text - DETX:

Estrogen **sulfotransferase** activity can be detected and quantified using an enzymatic assay. This assay can be used to measure EST activity in biological samples, preferably human samples, more preferably human liver preparations. It is sensitive and specific and can be used to detect the EST activity of any **sulfotransferase**, for example, human EST, DHEA ST and TS **PST**. The substrate used in the assay can be any estrogen or related chemical compound. Preferably, the substrate is estrone, estradiol-17.β., ethinyl estradiol or dehydroepiandrosterone (DHEA). More preferably, estrone is used as the sulfate acceptor substrate in the assay. The assay contains estrone and a magnesium (Mg.sup.2+) salt in a potassium phosphate buffer at a pH of about 6 to 7. Preferably, the assay mix contains 0.05 .μ.M estrone, 0.25 mM Mg.sup.2+ in 8.2 mM potassium phosphate buffer at pH 6.5.

Detailed Description Text - DETX:

Success was finally achieved using a direct PCR-based cloning strategy to obtain the full length human EST cDNA. 5'-and 3'-RACE were used to obtain sequences 5' and 3' to the ends of the 512 bp product. 5'-RACE was employed to obtain the sequence of the remainder of the 5'-end of the coding region as well as the 5'-untranslated region (UTR). The 5'-UTR of the cDNA consisted of 106 nucleotides (FIG. 1). 3'-RACE was used to obtain the sequence of the 3'-end of the ORF as well as the 3'-UTR (Table 1, FIG. 1). The full length human liver EST cDNA consisted of 1063 nucleotides with an 882 nucleotide ORF that encodes 294 amino acids (FIG. 2) (SEQ ID NO:1 and SEQ ID NO:2). The 3'-UTR included 72 nucleotides and ended in a poly(A) tract. The polyadenylation signal ATTAAA was located 24 nucleotides upstream from the poly(A) tract (FIG. 2). The sequence of the protein encoded by this human liver EST cDNA was 81, 73 and 72% identical with the amino acid sequences of guinea pig adrenocortical, bovine placental, and rat liver ESTs, respectively (FIG. 3A) (A. R. Nash et al., Aust. J. Biol. Sci., 41, 507-516 (1988); W. F. Demyan et al., Mol. Endocrinol., 6, 589-597 (1992); T. Oeda et al., Mol. Endocrinol., 6, 1216-1226 (1992)). However, it was only 37, 51 and 51% identical with the deduced amino acid sequences of human liver DHEA ST, TS **PST** and TL **PST**, respectively (FIG. 3A) (D. M. Otterness et al., Mol. Pharmacol., 41, 865-872 (1992); T. W. Wilborn et al., Mol. Pharmacol., 43, 70-77 (1993); T. C. Wood et al., Biochem. Biophys. Res. Commun., 198, 1119-1127 (1994)). Comparison of the deduced amino acid sequence of EST with those of 13 other ST enzymes showed many areas of sequence homology, two of which have been observed to be highly conserved throughout phylogeny. One of those sequences, YPKSGTXW (SEQ ID NO:16), is located toward the amino, and the other, RKGXXGDWKNXFT (SEQ ID NO:17), toward the carboxy terminus of the proteins. Comparisons of percentages of amino acid sequence identities (FIG. 3A) and a dendrogram depicting graphically the relationships among these proteins (FIG. 3B) confirmed that the STs are a gene superfamily with striking sequence identity among orthologous enzymes across species lines.

Detailed Description Text - DETX:

C. Results. The insert subcloned into pBluescript (Example I) contained the entire coding region of the human liver EST cDNA. The major in vitro translation product had an apparent relative molecular weight (M.sub.r) of 34.6 kDa estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE). The M.sub.r of human liver EST, calculated on the basis of amino acid sequence deduced from the sequence of the cDNA, was 35.1 kDa. ST activities were measured in preparations of transfected COS-1 cells using DHEA, 4-nitrophenol and dopamine, model substrates for DHEA ST, TS PST and TL PST, respectively. The protein encoded by the human liver EST cDNA was capable of catalyzing the sulfation of estrone (9 units/mg protein), DHEA (2.3 units/mg protein) and 4-nitrophenol (5 units/mg protein), but not that of dopamine. When transfection was performed with p91023(B) alone or with only buffer, no detectable ST activity was present with any of the substrates tested.

Detailed Description Text - DETX:

A. Sulfotransferase (ST) activities. The abilities of EST and DHEA ST to catalyze sulfation of various substrates were compared. ST activities were assayed by the method of Foldes and Meek (Biochem. Biophys. Acta., 327, 365-374 (1973)), as modified by Hernandez et al. (Drug Metab. Dispos., 20, 413-422 (1992)) for the measurement of human liver DHEA ST activity with its preferred substrate, DHEA, and by Campbell et al. (Biochem. Pharmacol., 36, 1435-1446 (1987)) for the measurement of human liver TS and TL PST activities with their preferred substrates, 4-nitrophenol and dopamine, respectively. These assays were performed under optimal conditions for the measurement of ST enzyme activities in human liver preparations (N. R. C. Campbell et al., Biochem. Pharmacol., 36, 1435-1446 (1987); J. S. Hernandez et al., Drug Metab. Dispos., 20, 413-422 (1992)). For the measurement of estrone sulfating activity, the optimized assay conditions described in Example III were used. Specifically, the sulfate acceptor substrates used in the various assays were DHEA (5 .mu.M), 4-nitrophenol (4 .mu.M), dopamine (60 .mu.M), and estrone (0.05 .mu.M), respectively. Controls did not contain sulfate acceptor substrates.

Claims Text - CLTX:

1. An isolated and purified DNA molecule comprising a DNA segment having a nucleotide sequence that encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2, or a biologically active fragment of said human estrogen sulfotransferase.

Claims Text - CLTX:

2. The DNA molecule of claim 1 wherein the DNA segment encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

4. The DNA molecule of claim 1 wherein the DNA segment encodes a biologically active fragment of a human estrogen sulfotransferase wherein the human estrogen sulfotransferase has the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

(a) a single coding region having a nucleotide sequence that encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2, or a biologically active fragment of said human estrogen sulfotransferase;

Claims Text - CLTX:

6. The DNA molecule of claim 5 wherein the single coding region encodes a human estrogen sulfotransferase having the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

8. The DNA molecule of claim 5 wherein the single coding region encodes a biologically active fragment of a human estrogen sulfotransferase wherein the human estrogen sulfotransferase has the amino acid sequence of SEQ ID NO:2.

Other Reference Publication - OREF:

I.A. Aksoy et al., "Cholesterol Sulfation in Human Liver: Catalysis by Dehydroepiandrosterone Sulfotransferase," Drug Metab. Dispos., 21, 268-276 (1993).

Other Reference Publication - OREF:

I.A. Aksoy et al., "Human liver dehydroepiandrosterone sulfotransferase: Nature and extent of individual variation", Clin. Pharmacol. Ther., 54, 498-506 (1993).

Other Reference Publication - OREF:

I.A. Askoy, et al., "Human Liver Estrogen Sulfotransferase: Identification by cDNA Cloning and Expression", Biochem. and Biophys. Research Commun. 200 1621-1629 (May 16, 1994).

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Liver Estrogen Sulfotransferase (EST): Polymerase Chain Reaction (PCR) Cloning of cDNA", (Abstract) ISSX Proc., 4, 181, 1993.

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Liver Estrogen Sulfotransferase (EST): Polymerase Chain Reaction (PCR) Cloning of cDNA", (Poster Presentation for the Fifth North American Meeting of the International Society for the Study of Xenobiotics) Oct. 18, 1993.

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Estrogen Sulfotransferase, cDNA Cloning, Expression and Characterization", (Abstract), Proc. Amer. Assoc. Cancer. Res., 35, 276 (1994).

Other Reference Publication - OREF:

I.A. Aksoy, et al., "Human Estrogen Sulfotransferase: cDNA Cloning, Expression, and Characterization", (Poster Presentation for the Eighty-Fifth Annual Meeting of the American Association for Cancer Research (AACR)) Apr. 9-13, 1994.

Other Reference Publication - OREF:

R.T. Borchardt, et al., "An Ecteola-Cellulose Chromatography Assay for 3'-Phosphoadenosine 5'-Phosphosulfate: Phenol Sulfotransferase," Anal. Biochem., 130, 334-338 (1983).

Other Reference Publication - OREF:

N.R.C. Campbell et al., "Human Liver Phenol Sulfotransferase: Assay Conditions, Biochemical Properties and Partial Purification of Isozymes of the Thermostable Form," Biochem. Pharmacol., 36, 1435-1446 (1987).

Other Reference Publication - OREF:

J.S. Hernandez et al., "Sulfation of Estrone and 17.β-Estradiol in Human Liver: Catalysis by Thermostable Phenol Sulfotranferase and by Dehydroepiandrosterone Sulfotransferase," Drug. Metab. Dispos., 20, 413-422 (1992).

Other Reference Publication - OREF:

W.B. Jakoby et al., "Sulfotransferases" in Enzymatic Basis of Detoxication; vol. II, W.B. Jakoby (ed), Academic Press: New York; pp. 199-229 (1980).

Other Reference Publication - OREF:

G.A. Johnson et al., "Sulfation of Minoxidil by Liver Sulfotransferase," Biochem. Pharmacol., 31, 2949-2954 (1982).

Other Reference Publication - OREF:

A-N.T. Kong et al., "Molecular Cloning of the Alcohol/Hydroxysteroid Form

(mST.sub.al) of **Sulfotransferase** from Mouse Liver," Pharmaceut. Res. 10, 627-630 (1993).

Other Reference Publication - OREF:

A-N.T. Kong et al., "Molecular Cloning of cDNA Encoding the **Phenol/Aryl Form of Sulfotransferase** (mST.sub.pl) from Mouse Liver," Biochem. Biophys. Acta, 1171, 315-318 (1993).

Other Reference Publication - OREF:

M.A. Mancini et al., "Spatio-Temporal Expression of Estrogen **Sulfotransferase** within the Hepatic Lobule of Male Rats: Implication of in Situ Estrogen Inactivation in Androgen Action," Endocrinology, 131, 1541-1546 (1992).

Other Reference Publication - OREF:

D.M. Otterness et al., "Human Liver Dehydroepiandrosterone **Sulfotransferase**: Molecular Cloning and Expression of cDNA," Mol. Pharmacol., 41, 865-872 (1992).

Other Reference Publication - OREF:

D.M. Otterness et al., "Human Liver Thermostable **Phenol Sulfotransferase**: Photoaffinity Labeling with 2-Iodo-4-Azidophenol," Mol. Pharmacol., 36, 856-865 (1989).

Other Reference Publication - OREF:

R.A. Price et al., "Genetic Polymorphism for Human Platelet Thermostable **Phenol Sulfotransferase** (TS **PST**) Activity," Genetics, 122, 905-914 (1989).

Other Reference Publication - OREF:

C. Reiter et al., "Thermolabile and Thermostable Human Platelet **Phenol Sulfotransferase**: Substrate Specificity and Physical Separation," Naunyn-Schmied. Archiv. Pharmacol., 324, 140-147 (1983).

Other Reference Publication - OREF:

R.D. Sekura et al., "Assay of **Sulfotransferases**," Analyt. Biochem., 95, 82-86 (1979).

Other Reference Publication - OREF:

R.S. Sundaram et al., "Human Intestinal **Phenol Sulfotransferase**: Assay Conditions, Activity Levels and Partial Purification of the Thermolabile Form," Drug Metab. Dispos., 17, 255-264 (1989).

Other Reference Publication - OREF:

L. Tseng et al., "Estrogen **Sulfotransferase** in Human Placenta," J. Steroid Biochem., 22, 611-615 (1985).

Other Reference Publication - OREF:

L. Varin et al., "Enzymatic Assay for Flavonoid **Sulfotransferase**," Anal. Biochem., 161, 176-180 (1987).

Other Reference Publication - OREF:

L. Varin et al., "Molecular Characterization of Two Plant Flavonol **Sulfotransferase**," Proc. Natl. Acad. Sci. USA, 89, 1286-1290 (1992).

Other Reference Publication - OREF:

R.M. Weinshilboum, "**Sulfotransferase** Pharmacogenetics," Pharmacol. Ther., 45, 93-107 (1990).

Other Reference Publication - OREF:

R.M. Weinshilboum et al., "**Sulfotransferase** Enzymes," in Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity; Handbook of Experimental Pharmacology series; F.C. Kauffman, Ed.; Springer-Verlag (1994).

Other Reference Publication - OREF:

R.M. Whittemore et al., "A Modified Ecteola Cellulose Assay for M and P **Phenol Sulfotransferase**," Biochem. Pharmacol., 34, 1647-1652 (1985).

Other Reference Publication - OREF:

T.W. Wilborn et al., "Sequence Analysis and Expression of the cDNA for the Phenol-Sulfating Form of Human Liver **Phenol Sulfotransferase**," Mol. Pharmacol., 43, 70-77 (1993).

Other Reference Publication - OREF:

T.C. Wood et al., "Human Liver Thermolabile Phenol Sulfotransferase: cDNA Cloning, Expression and Characterization," Biochem. Biophys. Res. Commun., 198, 1119-1127 (1994).

Other Reference Publication - OREF:

T. Oeda et al., "Molecular Cloning and Expression of a Full Length Complementary DNA Encoding the Guinea Pig Adrenocortical Estrogen Sulfotransferase", Mol. Endocrinol. 6(8) 1216-1226, Aug. 1992.

Other Reference Publication - OREF:

W.F. Demyan et al., "Estrogen Sulfotransferase of the Rat Liver: Complementary DNA Cloning and Age- and Sex-Specific Regulation of Messenger RNA", Mol. Endocrinol. 6(4) 589-597, Apr. 1992.

Other Reference Publication - OREF:

A.R. Nash et al., "Oestrogen Sulfotransferase: Molecular Cloning and Sequencing of cDNA for the Bovine Placental Enzyme", Aust. J. Bio. Sci. 41(4) 507-516, 1988.

Other Reference Publication - OREF:

T. Hondoh et al., "Purification and Properties of Estrogen Sulfotransferase of Human Fetal Liver", Biomedical Research 14(2) 129-136, Apr. 1993.

Other Reference Publication - OREF:

K.J. Forbes-Bamforth et al., "Identification of a New Adult Human Liver Sulfotransferase With Specificity for Endogenous and Xenobiotic Estrogens", Biochem. Biophys. Res. Commun. 198(2) 707-711, Jan. 1994.

US-PAT-NO: 5672584

DOCUMENT-IDENTIFIER: US 5672584 A

TITLE: Cyclic prodrugs of peptides and peptide nucleic acids having improved metabolic stability and cell membrane permeability

DATE-ISSUED: September 30, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchardt; Ronald T.	Lawrence	KS	N/A	N/A
Siahaan; Teruna	Lawrence	KS	N/A	N/A
Gangwar; Sanjeev	Lawrence	KS	N/A	N/A
Stella; Valentino J.	Lawrence	KS	N/A	N/A
Wang; Binghe	Norman	OK	N/A	N/A

APPL-NO: 08/ 429732

DATE FILED: April 25, 1995

US-CL-CURRENT: 514/11; 530/317

ABSTRACT:

Provided are cyclic prodrugs of biologically active peptides and peptide nucleic acids exhibiting improved cell membrane permeability and enzymatic stability, containing 3-(2'-hydroxy-4',6'-dimethyl phenyl)-3,3-dimethyl propionic acid and its derivatives and acyloxyalkoxy linkers. Also provided are pharmaceutical compositions containing effective amounts of these cyclic prodrugs in combination with pharmaceutically acceptable carriers, excipients, or diluents.

5 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

Baranczyk-Kuzma, A., Audus, K. L. and Borchardt, R. T. (1989) "Substrate specificity of phenol sulfotransferase from primary cultures of bovine brain microvessel endothelium," Neurochem. Res., 14, 689-691.

Detailed Description Text - DETX:

Baranczyk-Kuzma, A., Garren, J. A., Hidalgo, I. J. and Borchardt, R. T. (1991)
"Substrate specificity and some properties of phenol sulfotransferase from
human intestinal Caco-2 cells," Life Sci., 49, 1197-1206.

US-PAT-NO: 5580757

DOCUMENT-IDENTIFIER: US 5580757 A

TITLE: Cloning and expression of biologically active .alpha.-galactosidase A as a fusion protein

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Desnick; Robert J.	New York	NY	N/A	N/A
Bishop; David F.	New York	NY	N/A	N/A
Ioannou; Yiannis A.	New York	NY	N/A	N/A

APPL-NO: 08/ 261577

DATE FILED: June 17, 1994

PARENT-CASE:

This is a Divisional of Ser. No. 07/983,451, filed Nov. 30, 1992 now U.S. Pat. No. 5,401,650, which is a Continuation-In-Part of Ser. No. 07/602,824 filed Oct. 24, 1990 now U.S. Pat. No. 5,356,804, and Ser. No. 07/602,608 filed Oct. 24, 1990 now U.S. Pat. No. 5,382,524, each of which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 435/69.7; 435/208 ; 435/320.1

ABSTRACT:

The present invention involves the production of large quantities of human .alpha.-Gal A by cloning and expressing the .alpha.-Gal A coding sequence in eukaryotic host cell expression systems. The eukaryotic expression systems, and in particular the mammalian host cell expression system described herein provide for the appropriate cotranslational and posttranslational modifications required for proper processing, e.g., glycosylation, phosphorylation, etc. and sorting of the expression product so that an active enzyme is produced. In addition, the expression of fusion proteins which simplify purification is described.

Using the methods described herein, the recombinant .alpha.-Gal A is secreted by the engineered host cells so that it is recovered from the culture medium in good yield. The .alpha.-Gal A produced in accordance with the invention may be used, but is not limited to, in the treatment in Fabry Disease; for the hydrolysis of .alpha.-galactosyl residues in glycoconjugates; and/or for the conversion of the blood group B antigen on erythrocytes to the blood group O antigen.

15 Claims, 51 Drawing figures

Exemplary Claim Number: 5

Number of Drawing Sheets: 38

----- KWIC -----

Brief Summary Text - BSTX:

FIG. 28. Aggregation-Secretion Model for Selective Secretion of Human .alpha.-Gal A Overexpressed in CHO Cells. High level overexpression in CHO cells of human .alpha.-Gal A or other lysosomal enzymes which normally are targeted to the lysosome results in their selective secretion due to their aggregation and the resultant inaccessibility of their M6PR signals. The enzyme undergoes normal post-translational processing until it arrives in the TGN, where the overexpressed enzyme undergoes protein-protein interactions and forms smaller soluble and larger particulate aggregates, due to lower pH of the TGN. The TGN becomes dilated with the overexpressed enzyme. Some aggregates and soluble enzyme with exposed M6P signals are trafficked to lysosomes, while the majority of aggregates whose M6P are not accessible are exocytosed by default via the constitutive secretory pathway. In addition, decreased sulfation may occur as the tyrosines in the enzyme aggregates destined for secretion are unavailable to the sulfotransferase. This model may explain the selective secretion of other overexpressed proteins that normally are targeted to specific organelles.

Detailed Description Text - DETX:

Plasmid pcDAG126 (Bishop, et al., 1988, in, Lipid Storage Disorders, Salvaryre, R., Douste-Blazy, L. Gatt, S. Eds. Plenum Publishing Corporation, New York, pp. 809 to 822) containing the full-length .alpha.-Gal A cDNA was digested with Bam HI and Pst I and the 1.45 kb insert fragment was purified by agarose gel electrophoresis. The cDNA was then force-subcloned into plasmid pGEM-4 at the Bam HI and Pst I sites resulting in pGEM-AGA126. This plasmid was then digested with Hind III, end-filled using Klenow and ligated to Eco RI linkers. After digestion with Eco RI, the 1.45 kb fragment was purified as above and cloned into the Eco RI site of the mammalian expression vector p91023(B) (Wong et al., 1985, Science 228:810) resulting in p91-AGA (FIG. 1G).

Detailed Description Text - DETX:

Since it was conceivable that the overexpression of .alpha.-Gal A resulted in the formation of soluble and particulate aggregates that did not bind to or were inefficiently bound by the M6PR and/or the sulfotransferase in the TGN, the possible aggregation of .alpha.-Gal A was assessed in vitro at varying enzyme and hydrogen ion concentrations. As shown in FIG. 27A, the amount of .alpha.-Gal A precipitated, compared to about 30% (>2.times.10.sup.6 U) at pH 5.0. At pH 6.0, the estimated pH of the TGN (Griffith and Simons, 1986, Science 234: 438-443), about 12% of the enzyme formed particulate aggregates

that could be pelleted by centrifugation at 15,000.times.g. FIG. 27B shows that the turbidity, as a measure of aggregation (Halper and Stere, 1977, Arch. Biochem. Biophys. 184: 529-534), of solutions containing 0.1 to 10 mg/ml of .alpha.-Gal A at either pH 5.0 or 7.0 increased as a function of enzyme concentration. Moreover, the turbidity of a 1 mg/ml .alpha.-Gal A solution was essentially unaffected by the presence of increasing albumin concentrations from 0.1 to 10 mg/ml at pH 5.0 (FIG. 27B; control). Finally, electrophoresis of the supernatant and pellet fractions from solutions containing .alpha.-Gal A (10 mg/ml) and bovine serum albumin (BSA) (2 mg/ml) incubated at varying hydrogen ion concentrations revealed that the increasing precipitation of .alpha.-Gal A with decreasing pH was enzyme specific, as the BSA did not precipitate over this pH range (FIG. 27C).

US-PAT-NO: 5401650

DOCUMENT-IDENTIFIER: US 5401650 A

TITLE: Cloning and expression of biologically active .alpha.-galactosidase A

DATE-ISSUED: March 28, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Desnick; Robert J.	New York	NY	N/A	N/A
Bishop; David F.	New York	NY	N/A	N/A
Ioannou; Yiannis A.	New York	NY	N/A	N/A

APPL-NO: 07/ 983451

DATE FILED: November 30, 1992

PARENT-CASE:

This is a Continuation-In-Part of Ser. No. 602,824 filed Oct. 24, 1990 and Ser. No. 07/602,608 filed Oct. 24, 1990, each of which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 435/208; 435/193 ; 536/23.2 ; 536/23.4

ABSTRACT:

The present invention involves the production of large quantities of human .alpha.-Gal A by cloning and expressing the .alpha.-Gal A coding sequence in eukaryotic host cell expression systems. The eukaryotic expression systems, and in particular the mammalian host cell expression system described herein provide for the appropriate cotranslational and posttranslational modifications required for proper processing, e.g., glycosylation, phosphorylation, etc. and sorting of the expression product so that an active enzyme is produced. In addition, the expression of fusion proteins which simplify purification is described.

Using the methods described herein, the recombinant .alpha.-Gal A is secreted by the engineered host cells so that it is recovered from the culture medium in good yield. The .alpha.-Gal A produced in accordance with the invention may be used, but is not limited to, in the treatment in Fabry Disease; for the hydrolysis of .alpha.-galactosyl residues in glycoconjugates; and/or for the conversion of the blood group B antigen on erythrocytes to the blood group O antigen.

9 Claims, 51 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 38

----- KWIC -----

Drawing Description Text - DRTX:

FIG. 28. Aggregation-Secretion Model for Selective Secretion of Human α -Gal A Overexpressed in CHO Cells. High level overexpression in CHO cells of human α -Gal A or other lysosomal enzymes which normally are targeted to the lysosome results in their selective secretion due to their aggregation and the resultant inaccessibility of their M6PR signals. The enzyme undergoes normal post-translational processing until it arrives in the TGN, where the overexpressed enzyme undergoes protein-protein interactions and forms smaller soluble and larger particulate aggregates, due to lower pH of the TGN. The TGN becomes dilated with the overexpressed enzyme. Some aggregates and soluble enzyme with exposed M6P signals are trafficked to lysosomes, while the majority of aggregates whose M6P are not accessible are exocytosed by default via the constitutive secretory pathway. In addition, decreased sulfation may occur as the tyrosines in the enzyme aggregates destined for secretion are unavailable to the sulfotransferase. This model may explain the selective secretion of other overexpressed proteins that normally are targeted to specific organelles.

Detailed Description Text - DETX:

Plasmid pcDAG126 (Bishop, et al., 1988, in, Lipid Storage Disorders, Salvaryre, R., Douste-Blazy, L. Gatt, S. Eds. Plenum Publishing Corporation, New York, pp. 809 to 822) containing the full-length α -Gal A cDNA was digested with Bam HI and Pst I and the 1.45 kb insert fragment was purified by agarose gel electrophoresis. The cDNA was then force-subcloned into plasmid pGEM-4 at the Bam HI and Pst I sites resulting in pGEM-AGA126. This plasmid was then digested with Hind III, end-filled using Klenow and ligated to Eco RI linkers. After digestion with Eco RI, the 1.45 kb fragment was purified as above and cloned into the Eco RI site of the mammalian expression vector p91023(B) (Wong et al., 1985, Science 228:810) resulting in p91-AGA (FIG. 1C).

Detailed Description Text - DETX:

Since it was conceivable that the overexpression of α -Gal A resulted in the formation of soluble and particulate aggregates that did not bind to or were inefficiently bound by the M6PR and/or the sulfotransferase in the TGN, the possible aggregation of α -Gal A was assessed in vitro at varying enzyme and hydrogen ion concentrations. As shown in FIG. 27A, the amount of α -Gal A precipitated, compared to about 30% ($\approx 2 \times 10^6$ U) at pH 5.0. At pH 6.0, the estimated pH of the TGN (Griffith and Simons, 1986, Science 234:438-443), about 12% of the enzyme formed particulate aggregates that could be pelleted by centrifugation at 15,000 times g. FIG. 27B shows that the turbidity, as a measure of aggregation (Halper and Stere, 1977, Arch. Biochem. Biophys. 184:529-534), of solutions containing 0.1 to 10 mg/ml of

.alpha.-Gal A at either pH 5.0 or 7.0 increased as a function of enzyme concentration. Moreover, the turbidity of a 1 mg/ml .alpha.-Gal A solution was essentially unaffected by the presence of increasing albumin concentrations from 0.1 to 10 mg/ml at pH 5.0 (FIG. 27B; control). Finally, electrophoresis of the supernatant and pellet fractions from solutions containing .alpha.-Gal A (10 mg/ml) and bovine serum albumin (BSA) (2 mg/ml) incubated at varying hydrogen ion concentrations revealed that the increasing precipitation of .alpha.-Gal A with decreasing pH was enzyme specific, as the BSA did not precipitate over this pH range (FIG. 27C).